

Ancestral xerophobia: a hypothesis on the whole plant ecophysiology of early angiosperms

T. S. FEILD,¹ D. S. CHATELET¹ AND T. J. BRODRIBB²

¹*Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37919 USA*

²*Department of Plant Sciences, University of Tasmania, Hobart, Tasmania, Australia*

ABSTRACT

Today, angiosperms are fundamental players in the diversity and biogeochemical functioning of the planet. Yet despite the omnipresence of angiosperms in today's ecosystems, the basic evolutionary understanding of how the earliest angiosperms functioned remains unknown. Here we synthesize ecophysiological, paleobotanical, paleoecological, and phylogenetic lines of evidence about early angiosperms and their environments. In doing so, we arrive at a hypothesis that early angiosperms evolved in evermoist tropical terrestrial habitats, where three of their emblematic innovations – including net-veined leaves, xylem vessels, and flowers – found ecophysiological advantages. However, the adaptation of early angiosperm ecophysiology to wet habitats did not initially promote massive diversification and ecological dominance. Instead, wet habitats were permissive for the ecological roothold of the clade, a critical phase of early diversification that entailed experimentation with a range of functional innovations in the leaves, wood, and flowers. Later, our results suggest that some of these innovations were co-opted gradually for new roles in the evolution of greater productivity and drought tolerance, which are characteristics seen across the vast majority of derived and ecologically dominant angiosperms today.

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Corresponding author: Taylor S. Feild. Tel.: +1 865 974 4521; fax: +1 865-974-3067; e-mail: tfeild@utk.edu

INTRODUCTION

As today's vegetational dominants, angiosperm canopies, leaves, and roots provide metabolic interfaces that are fundamental to global terrestrial productivity and webs of ecological processes that are integral to functioning of in terrestrial biogeochemistry and diversity (Knoll & James, 1987; Volk, 1989; Robinson, 1990, 1994; Grimaldi, 1999; Berner, 2006). Yet beneath the deeply engrained roles of angiosperms in today's ecosystems lies a grand evolutionary enigma (Wing & Boucher, 1998). Although an ever-increasing number of new fossil discoveries and phylogenetic insights continues to illuminate the temporal dynamics and phylogenetic pattern of early angiosperm diversification (Friis *et al.*, 2006, 2007; Frohlich & Chase, 2007; Saarela *et al.*, 2007), the fundamental evolutionary questions about early angiosperm evolution resist understanding. Persistent uncertainties about early angiosperms include the identities of the gymnosperm clade that the angiosperms descended from, the timing of angiosperm origin and early diversification, as well as their ancestral form, function, and ecology and how these characteristics influenced the processes underpinning their

early evolutionary ascent in the Late Mesozoic (Stebbins, 1974; Doyle & Hickey, 1976; Hickey & Doyle, 1977; Retallack & Dilcher, 1981, 1986; Taylor & Hickey, 1996; Wing & Boucher, 1998; Feild *et al.*, 2004; Feild & Arens, 2005, 2007; Bateman *et al.*, 2006; Doyle, 2006; Friis *et al.*, 2006, 2007; Frohlich & Chase, 2007; Zavada, 2007; Endress & Doyle, 2009).

Our aim here is to synthesize recent phylogenetic, ecophysiological, and geological evidence toward an understanding one of these uncertainties – how the earliest angiosperms functioned in relation to their habitats. In doing so, we frame a new hypothesis for the ancestral ecophysiological context of angiosperms. Our new hypothesis – hereafter called the 'xerophobia hypothesis' – proposes that the earliest angiosperms were fundamentally drought intolerant and required high and dependable moisture supplies to function. Our proposal builds upon earlier comparative work suggesting that early angiosperms first explored damp and shady, disturbed microsites in forest understories (Feild *et al.*, 2004; Feild & Arens, 2005, 2007; Feild, 2008). However, here we focus largely on water for two reasons. First, our presented analysis shows that water emerges the most important resource constraining the

ecophysiological performances of extant basal angiosperm lineages that diverged early in angiosperm evolution. Second, we show that there are several lines of fossil evidence suggesting that wet habitat adaptation is represented in the earliest known angiosperm fossils.

We begin by reviewing and providing new data on the comparative climatic ties and water balance ecophysiology of extant early diverging angiosperm lineages. These results motivate our proposal of the xerophobia hypothesis. Then, we show how an understanding of water balance physiology among extant basal angiosperm clades offers new hypotheses on the ancestral functions and evolutionary selective pressures associated with early evolution of innovations that define the fundamental body plan of angiosperms. We also examine how these traits were later co-opted for diversification in a wider range of environments. The innovations of concern here are densely veined leaves, xylem vessels, and flowers. We focus on these features because they define the tremendous productivities and rapid reproductive rates that are achieved by angiosperms. These functional traits are also responsible for unique angiosperm overprint on how terrestrial biogeochemical cycles function today as compared to pre-angiosperm plant communities (Stebbins, 1974; Carlquist, 1975; Doyle & Donoghue, 1986; Knoll & James, 1987; Bond, 1989; Volk, 1989; Robinson, 1990, 1994; Grimaldi, 1999; Berner, 2006; Williams, 2008; CK Boyce, TJ Brodribb, TS Feild, MA Zwieniecki, in review). Next, we reconsider the paleoecology of the early angiosperm fossil record to examine if there are signs of wet-adapted early angiosperms in the fossil record. After finding preliminary evidence for wet habitat adaptation in the Early Cretaceous angiosperm fossil record, we conclude with a brief discussion of how water economy possibly shaped the early diversification of angiosperms.

EXTANT EVIDENCE FOR ANCESTRAL XEROPHOBIA AND THE EARLY EVOLUTION OF WATER USE BY LEAVES, WOOD, AND FLOWERS

Phylogenetic context

Molecular phylogenetic studies based on diverse molecular markers and genomic evidence from a broad range of angiosperms have recast our understanding of angiosperm relationships. These studies support the lineages of *Amborella* (one species), Nymphaeales [including *Tritburia* (Hydatellaceae), formerly supposed to be monocots; about 70 species], and Austrobaileyales (about 300 species) as a grade of 'basal most' lineages diverging before the origin of core angiosperms (the Mesangiospermae of Cantino *et al.*, 2007) – the clade representing the vast majority of angiosperms (Fig. 1). Recent analyses of nearly complete plastid genomes have begun to resolve relationships of clades branching above the robustly supported basal grade. These studies suggested that a clade

containing the Magnoliidae (as Cantino *et al.*, 2007, referred to here as magnoliids, which includes Magnoliales, Laurales, Winterales, and Piperales) and the Chloranthaceae branched next (Jansen *et al.*, 2007; Moore *et al.*, 2007). Monocotyledoneae (referred to as monocots) and a clade containing Eudicotyledoneae (referred to as eudicots) diverged above the Magnoliidae-Chloranthaceae clade. In some studies, the highly reduced aquatic lineage *Ceratophyllum* was supported as the sister group of eudicots (Jansen *et al.*, 2007; Moore *et al.*, 2007). However in other studies, Chloranthaceae branched immediately above the grade of basal most lineages (Doyle & Endress, 2000; Saarela *et al.*, 2007). Or in other studies, Chloranthaceae were supported as the sister group to monocots (Qiu *et al.*, 2005). *Ceratophyllum* was also placed as the sister group of Chloranthaceae in some studies (see Endress & Doyle, 2009). Because of the unknown identity of the gymnosperm outgroups to angiosperms (Bateman *et al.*, 2006; Doyle, 2006, 2008; Friis *et al.*, 2007; Endress & Doyle, 2009), the resolution of the basal most extant angiosperm relationships offers a living comparative system for proposing hypotheses on diverse aspects of early angiosperm botany.

Below *Amborella*, Nymphaeales, and Austrobaileyales will serve as the comparative framework of basal angiosperm lineages for inferring the whole plant biology of water regulation in early angiosperms. In our analysis, we emphasize how physiological functions relate to structure as anatomy can provide mechanistic lifelines for reviving ancient early angiosperm physiologies from fossils. We also include Chloranthaceae in our analyses and discussions because (i) the lineage is possibly the sister group to magnoliids or may diverge immediately above the well-supported basal grade, (ii) the lineage shares a high number of plesiomorphic morphological traits with *Amborella* and Austrobaileyales, (iii) the oldest known fossils of angiosperms that can be associated with a living angiosperm subgroup are chloranthids, (iv) chloranthids appear to be the first angiosperm lineage to colonize high-latitude regions in both hemispheres, and (v) chloranthids were probably the first worldwide-distributed angiosperm lineage (Walker & Walker, 1984; Pedersen *et al.*, 1991; Brenner, 1996; Eklund *et al.*, 2004; Jansen *et al.*, 2007; Endress & Doyle, 2009).

Hydrological envelopes and habitats

In the field, seekers of basal angiosperms will immediately experience the hydrological essence of the xerophobia hypothesis. From standing on the muck-embedded *Nuphar* water lily rhizomes while chest deep in a pond, to dodging raining leeches during a montane ascent to reach *Austrobaileya* vines, and climbing over gnarled elfin trees with fog-drenched mossy branches in a hunt for *Hedyosmum* in tropical montane forests bedecked by clouds – basal angiosperm hunters are going to get very wet when looking for these plants. To add a comparative dimension to the field-evident experience, we conducted a phylogenetic analysis of the climatic envelope

variation as defined by Holdridge life zone (Fig. 1). Three fundamental aspects of regional climate parameterize Holdridge life zones: mean annual temperature, mean annual precipitation, and potential evapotranspiration (Holdridge, 1967). These variables define the broad limiting conditions for ecosystem function, and each life zone is named to suggest a generalized vegetation formation. However, local scale variations in habitat physiognomy and disturbance history within a life zone cannot be specified.

Our phylogenetic analysis of basal most lineages, including Chloranthaceae, indicated that the common ancestor of extant angiosperms occurred in either a subtropical lower montane rainforest or subtropical lower montane wet forest life zone (Fig. 1). Hereafter, we group these two formations together simply as lower montane subtropical forest (LMSF) life zones because these categories differ slightly from each other (Holdridge, 1967). LMSF life zones occur in low latitudes and upland regions, typically 1000 to 1800 m a.s.l. LMSFs experience high rainfalls (2000 to 4000 mm per year) and moderate mean annual temperatures (12 to 17 °C; Holdridge, 1967; Bruijnzeel, 2001). A characteristic hydrological feature of LMSF life zones is the regular and prolonged immersion in cloud. Cloud immersion greatly lowers evapotranspirational demand that a plant canopy experiences through decreasing solar insolation and increasing humidity. Thus, plants in these environments experience a narrow envelope of low leaf-to-air vapor pressure deficits (LAVPD; Bruijnzeel, 2001; Foster, 2001). Also, wind-blown cloud droplets that condensate on plant canopies and drip into the soil bolster moisture availability. Although analogizing cloud moisture to rainfall is difficult, estimates suggested that cloud water inputs range from equivalent to 300 to 1500 mm of additional rainfall a year (Bruijnzeel, 2001; Foster, 2001). LMSF life zones are among the terrestrial vegetation types experiencing the lowest amounts of drought stress possible.

Above the nodes parameterized by basal most angiosperms, the ancestral node of Magnoliidae was reconstructed as in the LMSF life zone. The eudicots, however, were reconstructed as ancestrally from a wet warm temperature forest life zone. The ancestral nodes of monocots and *Ceratophyllum*, although not in the LMSF life zone, are both reconstructed as freshwater

aquatic herbs (Fig. 1). Nymphaeales also represent a lineage of derived aquatics (Fig. 1). However, these shifts to the aquatic habit are consistent with the xerophobia hypothesis because these lineages function as extreme hydrophytes (Feild & Arens, 2005, 2007).

At a more local scale, a hypothesis for the ancestral habitat of early angiosperms has also been retrodicted from living plants. The phylogenetic patterning of seedling habitats as well as the ecophysiological and ecomorphic traits (see succeeding discussion) across extant basal clades indicated that their common ancestor was a small tree or shrub that occurred in well-drained, disturbed microsites of the wet forest understory (Feild *et al.*, 2004; Feild & Arens, 2005, 2007; Feild, 2008). Examples of these 'damp, dark, and disturbed' habitats include unstable sandy ravine sides eroded by meandering streams, tip-up mineral soil mounds formed in the aftermath of small tree falls, and moss mat crevices on rotting logs and tree fern trunks – all moist microsites heavily shaded by canopy trees. These sites experience diffuse light intensities ranging from 2% to 15% of full sunlight. Thus, compounding the high moisture availability of the cloud forest, the common ancestor reconstructed from living plants occurred in some of the wettest and least evaporative zones within everwet vegetation types.

Leaf water balance and structure

As the primary interface between plants and the atmosphere, leaves are the best place to begin a consideration of the capacity for whole plant water use and its regulation. Here we use the leaf characteristics of extant basal angiosperms to consider: what was the water use physiology of the ancestral angiosperm leaf, and what structural features set its performance?

The phylogenetic pattern of physiological traits parameterizes the ancestral node with a maximal stomatal conductance (g_{sMax}) of 0.169 mol H₂O m⁻² s⁻¹ and a photosynthetic rate (A_{Max}) of 7.52 μmol CO₂ m⁻² s⁻¹ under optimum physiological conditions and with the Nymphaeales excluded from the analysis (Table 1). The exclusion of the Nymphaeales from phylogenetic inferences on the ancestral states is justifiable for inferring early evolution of angiosperm water use because their

Table 1 Ancestral-state value estimates of ecophysiological traits of leaf and stem performance for extant basal angiosperms. Values for the reconstructed common ancestor of extant angiosperms are reconstructed by square-change parsimony in Mesquite version 2.6 (Maddison & Maddison, 2008). The data matrix for ancestral state reconstructions of leaf function [maximum CO₂ assimilation rate (A_{max} , μmol CO₂ m⁻² s⁻¹) on leaf area basis; maximum stomatal conductance to water vapor (g_{sMax} , mol H₂O m⁻² s⁻¹); water potential at the turgor loss point (TLP, MPa)] and anatomy [leaf venation density (VD) mm mm⁻²] are presented in Table 2. Stem performance traits [maximum stem hydraulic capacity (K_s , kg H₂O m⁻¹ s⁻¹ MPa⁻¹); tension inducing a 50% loss of stem hydraulic conductivity (PLC50%, MPa)] were taken from the literature (Sperry *et al.*, 2007). Values for the common ancestor were reconstructed by the squared-change parsimony option for continuous characters in Mesquite. Estimates are shown for the phylogenetic hypothesis of basal most lineages (*Amborella*, Nymphaeales, Austrobaileyales as well Chloranthaceae) that is depicted in Fig. 1

Node	A_{max}	g_{sMax}	TLP	VD	K_s	PLC50%
Common ancestor of extant angiosperms (-Nymphaeales)	7.52	0.169	-1.1	3.84	0.61	-2.5
Common ancestor of extant angiosperms (+Nymphaeales)	10.46	0.239	-1.1	3.69	0.61	-2.5

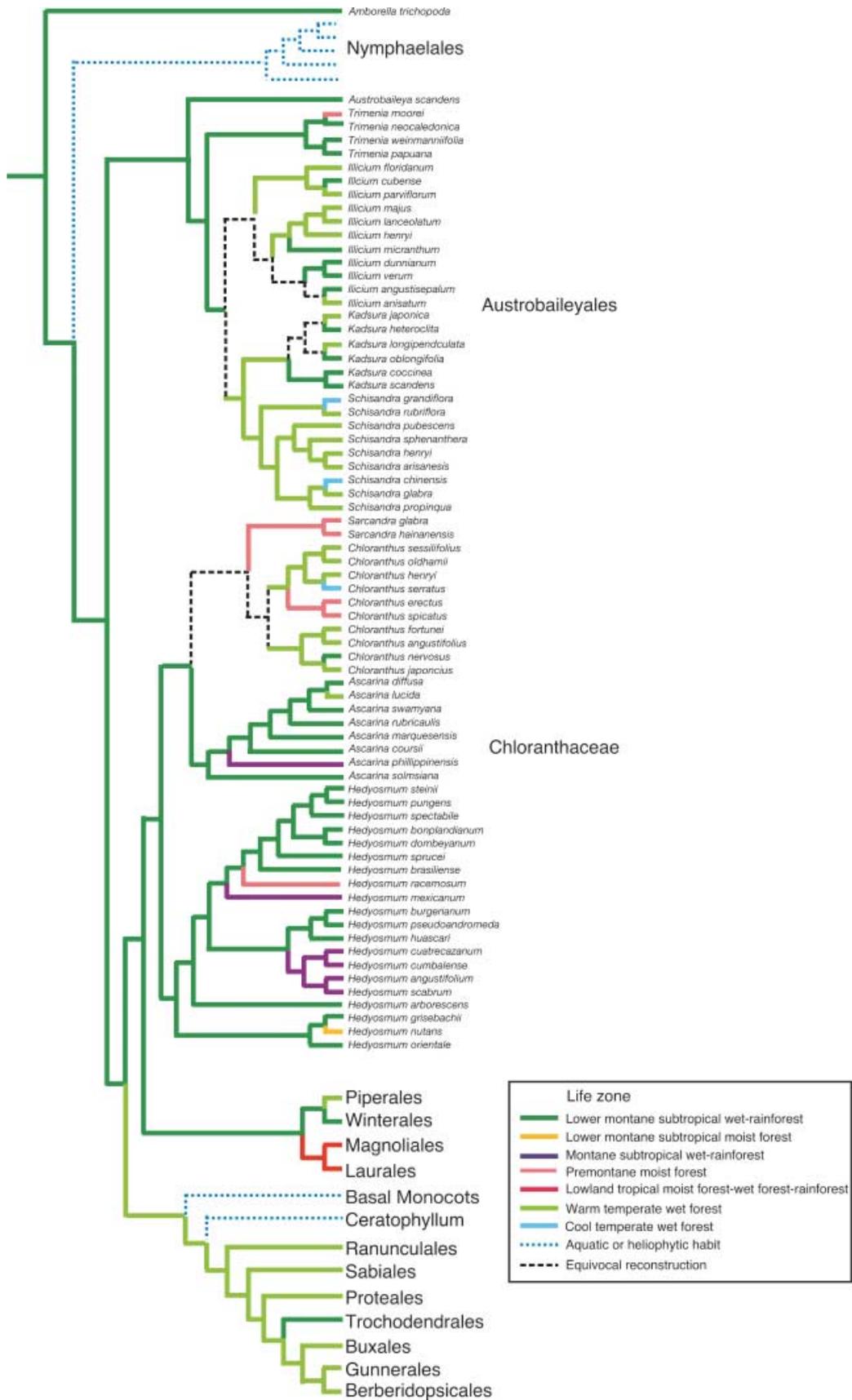


Table 2 Values for quantitative traits for extant basal most angiosperm species used in the ancestral reconstruction depicted in Fig. 2. Abbreviations for variables are as defined in Table 1. Maximum CO₂ assimilation rate and maximum stomatal conductance to water vapor were measured using the infrared gas-analysis as described previously and using accepted techniques (Feild *et al.*, 2003b, 2004; Brodribb *et al.*, 2007). The water potential at the turgor loss point (TLP, MPa) was inferred from pressure-volume curves using the methods and analyses described by Sack and colleagues (2003). Leaf vein density (VD, mm/mm²) was determined from portion of leaves cleared in sodium hydroxide (10% aqueous) and stained in safranin. Stained veins were traced on digital images of cleared leaves that were photographed using a digital camera at 50 x. Trait values for K_s and PLC50% variables are reported in Sperry *et al.* (2007). Samples sizes the variables are: A_{max} and g_{sMax}, ten leaves from ten individual plants; TLP, seven leaves from seven individual plants; VD, five measurements from five leaves. Error denotes the standard deviation around the mean. Values for TLP for Nymphaeales were not available (NA) because petiole aerenchyma precluded determination of PV curve

Species	A _{max} (SD)	g _{sMax} (SD)	TLP (SD)	VD
<i>Amborella trichopoda</i>	7.90 ± 0.15	0.201 ± 0.015	-1.1 ± 0.2	4.32 ± 0.23
<i>Ascarina rubricaulis</i>	10.3 ± 0.1	0.225 ± 0.022	-1.4 ± 0.3	4.60 ± 0.12
<i>Ascarina solmsiana</i>	6.12 ± 0.05	0.075 ± 0.010	-0.75 ± 0.1	2.35 ± 0.1
<i>Austrobaileya scandens</i>	4.29 ± 0.15	0.055 ± 0.005	-1.3 ± 0.2	2.20 ± 0.05
<i>Brasenia scherberi</i>	22.35 ± 1.1	0.435 ± 0.035	NA	1.23 ± 0.04
<i>Chloranthus erectus</i>	4.77 ± 0.3	0.066 ± 0.006	-0.675 ± 0.1	2.03 ± 0.06
<i>Chloranthus japonicus</i>	8.37 ± 0.6	0.149 ± 0.013	-0.75 ± 0.3	3.09 ± 0.15
<i>Chloranthus serratus</i>	6.46 ± 0.1	0.112 ± 0.011	-0.6 ± 0.1	2.24 ± 0.16
<i>Chloranthus spicatus</i>	4.45 ± 0.15	0.079 ± 0.008	-0.75 ± 0.2	3.41 ± 0.1
<i>Hedyosmum bonplandianum</i>	10.7 ± 0.23	0.160 ± 0.005	-1.1 ± 0.2	3.06 ± 0.12
<i>Hedyosmum brenesii</i>	8.37 ± 0.2	0.170 ± 0.014	-0.85 ± 0.1	2.94 ± 0.1
<i>Illicium anisatum</i>	7.06 ± 0.15	0.088 ± 0.004	-1.35 ± 0.2	3.78 ± 0.1
<i>Illicium floridanum</i>	8.83 ± 0.1	0.116 ± 0.014	-1.1 ± 0.4	4.92 ± 0.25
<i>Illicium henryi</i>	7.35 ± 0.1	0.099 ± 0.008	-1.0 ± 0.1	3.67 ± 0.21
<i>Illicium parviflorum</i>	10.91 ± 0.23	0.193 ± 0.015	-1.5 ± 0.2	5.71 ± 0.31
<i>Kadsura japonica</i>	6.53 ± 0.21	0.100 ± 0.009	-1.1 ± 0.3	3.45 ± 0.23
<i>Kadsura longipendunculata</i>	9.86 ± 0.1	0.209 ± 0.020	-1.2 ± 0.3	5.02 ± 0.35
<i>Nuphar polysepalum</i>	21.60 ± 0.16	0.647 ± 0.054	NA	5.85 ± 0.33
<i>Nymphaea odorata</i>	23.56 ± 1.2	0.449 ± 0.045	NA	0.46 ± 0.1
<i>Sarcandra glabra</i>	4.42 ± 0.13	0.058 ± 0.009	-0.85 ± 0.1	2.83 ± 0.15
<i>Schisandra arisanensis</i>	10.13 ± 0.23	0.180 ± 0.045	-1.1 ± 0.1	4.52 ± 0.14
<i>Schisandra glabra</i>	8.19 ± 0.25	0.133 ± 0.013	-0.85 ± 0.2	3.41 ± 0.21
<i>Schisandra henryi</i>	7.77 ± 0.21	0.118 ± 0.018	-0.95 ± 0.4	3.19 ± 0.1
<i>Schisandra propinqua</i>	6.57 ± 0.15	0.161 ± 0.023	-1.1 ± 0.4	4.11 ± 0.31
<i>Trimenia neocaledonica</i>	7.29 ± 0.1	0.157 ± 0.022	-1.15 ± 0.3	3.76 ± 0.28

derived and extensive ecophysiological divergence from other basal clades associated with adaptation to aquatic habitats (i.e. loss of stomata, high water loss rates associated with water-unlimited habitats). The g_{sMax} and A_{Max} values of most basal angiosperms fall within the ranges of moderately low capacities found for shade-tolerant forest understory and subcanopy

woody species from tropical to temperate zones worldwide (Feild *et al.*, 2001, 2004; Feild & Arens, 2005, 2007; Brodribb *et al.*, 2007). Thus, widespread occurrence of low A_{Max} values among extant taxa suggests that the evolution of high productivity occurred later in angiosperm history, except in the aquatic environment (Table 2).

Fig. 1 Phylogenetic character mapping of Holdridge life zones (Holdridge, 1967) among extant basal angiosperms. The composite phylogenetic tree was assembled by grafting several published phylogenetic hypotheses onto a backbone topology. Selection criteria for the consensus phylogenies used in the reconstruction are as described previously (Feild *et al.*, 2004). For the phylogenetic backbone, we used the results of Moore *et al.* (2007) analyses of basal angiosperm phylogeny. Patterns of Holdridge life zones evolution were reconstructed using Mesquite version 2.6 (Maddison & Maddison, 2008). The life zone character was treated as unpolarized and unordered. The most parsimonious reconstruction is depicted. The lineages were coded by seven life zone categories. Aquatics and heliophytes were coded separately as these ecologies operate under essentially unlimited access to water. Based on the species that are represented in phylogenetic studies, we coded each species into one of the seven life zones. If a species occurred in more than one life zone, the life zone representing the drier end of the hydrological range was used in the coding. For clarity of presentation, these results at the species tips were pruned, and only the ancestral states for major clades are depicted. For a details and references on the life-zone coding for clades, a manuscript is forthcoming on environmental niche envelop evolution among extant basal angiosperm lineages. Phylogenies from the literature that were used included the following: Austrobaileales: Schisandraceae Hao *et al.*, 2001; *Illicium* Morris *et al.*, 2007; Chloranthaceae: Zhang & Renner, 2003; Eklund *et al.*, 2004; Berberidopsidales: Soltis *et al.*, 2005; Buxales: von Balthazar *et al.*, 2000; Eudicots backbone: Worberg *et al.*, 2007; Gunnerales: Wanntorp *et al.*, 2002; Laurales general backbone Renner & Chanderbali, 2000; Atherospermataceae: Renner *et al.*, 2000; Calycanthaceae: Zhou *et al.*, 2006; Hernandiaceae: Renner & Chanderbali, 2000; Lauraceae: Chanderbali *et al.*, 2001; Monimiaceae: Renner & Chanderbali, 2000; Siparunaceae: Renner & Won, 2001; magnoliales: general backbone including Eupomatiaceae, Degeneriaceae, and Himantandraceae: Doyle *et al.*, 2004; Annonaceae: Doyle *et al.*, 2004; Myristicaceae: Sauquet *et al.*, 2003; Magnoliaceae: Azuma *et al.*, 2001; Nymphaeales: Borsch *et al.*, 2007; Piperales: Wanke *et al.*, 2007a,b; Proteales: Proteaceae: Jordan *et al.*, 2008; Platanaceae: Grimm & Denk, 2008; Ranunculales Berberidaceae: Kim *et al.*, 2004; Lardizabalaceae: Hoot *et al.*, 1995; Menispermaceae: Jacques & Bertolino, 2008; Papaveraceae: Hoot *et al.*, 1997; Ranunculaceae: Ro *et al.*, 1997; Sabiales: Worberg *et al.*, 2007; Trochodendrales: Worberg *et al.*, 2007; Winterales: Karol *et al.*, 2000.

In addition, tissue pressure–volume relations of basal angiosperm leaves indicate that optimum photosynthetic performance can only occur in a narrow range of water deficit. The wilting point (or turgor loss point, TLP) of the ancestral angiosperm leaf occurred at leaf water potential (Ψ_{leaf}) of -1.1 MPa. The average TLP for mesic woody plants, including other cloud forest species, lies between -2 and -3 MPa (Kapos & Tanner, 1985; Cavelier, 1990; Sack *et al.*, 2003; Lenz *et al.*, 2006). The wilting point is significant because once reached the stomata close and any significant productivity is halted. Turgor loss and stomatal closure at such high Ψ_{leaf} values place extant basal angiosperms as among the most susceptible of all woody plants to water limitation. Such extreme sensitivity of productivity to water availability means that basal angiosperms are physiologically capable of inhabiting only the most reliably moist habitats.

Anatomically, phylogenetic mapping of leaf ecoanatomical traits across extant basal angiosperms returns a reconstruction of the ancestral leaf with very low vein density (3.84 mm mm^{-1} ; Table 1). Vein densities of extant basal angiosperms (Table 2) are two to five times lower than those of the vast majority of extant eudicot and monocot angiosperms (Brodribb *et al.*, 2007). Vein densities of extant basal most angiosperms nest within the small range of low values found in ferns, lycopods, and several nonangiosperm seed plant lineages that are both living and extinct (CK Boyce, TJ Brodribb, TS Feild, MA Zwieniecki, in review). The presence of these low vein densities is a key finding by placing functional limits on the water use characteristics of the reconstructed common ancestor of extant angiosperms. Specifically, leaves with low vein density (but water lilies are exceptions, see succeeding discussion) are physically unable to support high rates of transpiration and photosynthesis because water transport from the terminal orders of the vein system to the sites of evaporation is very inefficient (Sperry, 2003; Brodribb *et al.*, 2007). Vein spacing largely sets a leaf's liquid-phase leaf hydraulic-transport capacity because the braided and ramified networks of microporous and water-filled tracheary elements of veins furnish much lower resistances to water flow than living parenchymatous tissue (Brodribb *et al.*, 2007). If the flow path through the mesophyll is long (as it will be when vein density is low), then even moderate rates of transpiration will generate a large negative Ψ gradient in the leaf, leading to turgor loss and stomatal closure (Brodribb *et al.*, 2007).

Another ancestral leaf trait that signals low tolerance to drought is the presence of chloranthoid teeth along the edge of the leaf blade (Feild *et al.*, 2003a, 2005; Feild & Arens, 2007). Experimental work on *Chloranthus japonicus* (Chloranthaceae) demonstrated that chloranthoid teeth participate in the regulation of root pressure. In the absence of transpiration, root pressure occurs by what appears to be a reverse osmosis process, where ions are actively loaded into the root to attract water and thereby create a positive xylem hydrostatic pressure (Kramer & Boyer, 1995). As root pressure rises, water flows

upward from the root system, through the stem xylem, and eventually to the leaf venation system. Chloranthoid teeth regulate leaf pressurization by bleeding off excess pressure through regulating the release of water – a process called guttation. When teeth are experimentally blocked, root-pressure-driven water flow has nowhere to go, and water backflows into the leaf. The resulting drowning of the mesophyll air spaces by guttation sap inhibits photosynthesis by slowing CO_2 diffusion (Feild *et al.*, 2005). The role of chloranthoid teeth in bleeding off root-pressurized sap and therefore avoiding possible ill physiological effects is widespread among basal angiosperms. Field guttation and root pressure have been observed from the leaf teeth of *Amborella*, *Trimenia*, and Schisandraceae, all genera of Chloranthaceae, as well as several woody and herbaceous basal eudicot lineages that retain them such as *Euptelea*, *Tetracentron*, Ranunculaceae, and mesic-adapted Berberidiaceae and Papaveraceae (TS Feild, unpublished observations, 2003–2008).

Because precious little water can be lost from terrestrial basal angiosperm leaves before wilting, and root pressure requires metabolic energy, why do leaves of many species so frequently release water that is decoupled from transpiration and productivity? Textbooks tend to treat root pressure and guttation as quaint botanical curiosities rather than as ecophysiological significant processes (Kramer & Boyer, 1995). However, increasing evidence suggests that root pressure is important (Feild *et al.*, 2005; Clearwater *et al.*, 2007; Cobb *et al.*, 2007). For example in evermoist habitats that often experience near saturating humidity, such as in the forest understory, in streamside zones, or in cloud forests, root pressure can act as a reliable and effective mechanism to recover rapidly from drought-stress-induced embolism of the xylem. This is important because basal angiosperms appear to be especially vulnerable to xylem cavitation (see succeeding discussion). Also, root pressure has been shown to elevate night-time shoot turgor and/or maintain the delivery of solutes and hormones dissolved in the xylem sap to growing shoots when transpiration is suppressed (Feild *et al.*, 2005; Clearwater *et al.*, 2007; Feild & Arens, 2007). Both processes are important in the regulation of growth in leaves, flowers, and fruits. Consistent with functional coordination among root pressure, guttation, and growth, other growing organs besides leaves, such as stipules and floral parts, often bear guttating chloranthoid teeth or epithem glands (Feild & Arens, 2007).

Early evolution of the angiosperm leaf

The phylogenetic distributions of extant basal angiosperm ecologies indicate that the initial evolutionary exploration of complex vein branching of the venation system occurred in a very wet environment (Fig. 1). Wet environments may have been permissive for early experiments in vein reticulation by offering low Ψ variation. Experiencing low drought stress is important because extant basal most angiosperms suggest that the initial vein densities of these experiments were low, and the

mesophyll tissues wilted at modestly low Ψ (Table 1; Table 2). Vein reticulation may have offered advantages to angiosperms in their reconstructed ancestral niche as forest understory plants (Feild *et al.*, 2004; Feild & Arens, 2005, 2007). For example, finely reticulated veins permit the evolution of broad, undissected laminae that are important in optimizing light-use efficiency in the understory as compared to laminae that are narrow and dissected. Reticulated veins homogenize the distribution of vein density and therefore lessen gradients in hydraulic supply across the leaf (Brodribb *et al.*, 2007). Such a design permits a large photosynthetic surface that also operates with a more uniform distribution of Ψ across the leaf during transpiration. More uniform Ψ in the leaf may be important in increasing the efficiency of harvesting sunflecks by avoiding the closing of the stomata too soon. Sunflecks provide the bulk of the diurnal subsidy of light to forest understory plants. Because light cannot be used for carbon assimilation without losing water, effective harvesting of sunflecks necessitates reasonable hydraulic transport in the leaf because sunflecks generate surges in transpiration as the leaf heats up. This is especially the case if the photosynthetic surface area is large, which in the first approximation would be expected to be the optimal form for the capture of sunflecks (Leakey *et al.*, 2005; Smith *et al.*, 1997).

The complex vein reticulation found in angiosperm leaves we suggest enabled new abilities to exploit sunflecks as compared to most nonangiosperm plants. Most extant and extinct lycopods, ferns, and gymnosperms possess leaves that include (i) a single vascular strand, (ii) bifurcated veins in otherwise parallel vein systems of low vein density, (iii) reticulation that tends to be clustered on the edge of the lamina or that is of low density (Wagner, 1977; Boyce, 2005; Brodribb *et al.*, 2007; CK Boyce, TJ Brodribb, TS Feild, MA Zwieniecki, in review). Unlike the leaves of extant basal most angiosperms, these designs cannot simultaneously deliver a uniform Ψ over the lamina whereas providing a high surface of photosynthetic area that is enabled by complexly veined angiosperm leaves. Thus, we would hypothesize that many understory lycopods (Zwieniecki *et al.*, 2002), ferns, and gymnosperms would have lower sunfleck use-efficiencies as compared to extant basal most angiosperms in the field. Interestingly, some of the other instances of nonangiosperms that have evolved complex reticulated vein systems likely occurred under wet, shady conditions. Examples of these clades include *Gnetum*, the marattioid fern *Christensenia*, several Polypodiaceae ferns, and possibly one or more lineages within the extinct seed plants gigantopterids (Boyce, 2005; Feild & Balun, 2008). However, other nonangiosperms lineages may have evolved complex reticulation in higher light intensity environments. A specific example is *Dipteris* (Dipteridiaceae) ferns, which are shade intolerant colonizers of bare landslips in tropical cloud forests.

Only in the angiosperms does it appear to be the case that complex reticulated veins were exported to a broader range of environments (Boyce, 2005). Indeed today, the familiar

highly reticulated venation system of the angiosperm leaf is responsible for productivity and transpiration of the most productive and species diverse canopies in the world. A key event toward the ecological ability of vein reticulation in angiosperms appears to be at least partially related to the unique ability of angiosperms to greatly increase vein density in the leaf. CK Boyce and colleagues (in press) demonstrated that during the 380-million-year history of laminate leaves, nonangiosperms average only 3.5 mm mm⁻², whereas vein densities ranging from 7 to almost 25 mm mm⁻² densities are found in most angiosperms. Whereas densely veined leaves are unique to the angiosperms, the phylogenetic distribution of sparsely veined leaves (<5 mm mm⁻²) among basal most angiosperm lineages suggests that the evolution of high vein density was not acquired with the origin of the extant angiosperm crown group (Table 2). Instead, high vein density probably arose independently and gradually in the magnoliids, monocots, and eudicots (CK Boyce, TJ Brodribb, TS Feild, MA Zwieniecki, in review).

The evolution of high leaf vein density was a breakthrough that allowed angiosperms to achieve maximum leaf transpiration rates and productivities never before available to any other clade, including the few clades that evolved angiosperm-like reticulate venation (Brodribb *et al.*, 2007). Because the leaf mesophyll tissue is specialized for the biochemical, gaseous, and optical transport processes associated with photosynthesis rather than for hydraulic flow, high vein density means that water can be delivered from the vein to close to the sites of evaporation near stomata pores. These benefits conveyed to the leaf in terms of hydraulic efficiency, which are translated almost proportionally into increased photosynthetic yield and transpirational capacity (Brodribb *et al.*, 2007). By allowing leaves to transpire at a rapid rate with minimal desiccation, the plant profits from an increased yield in the exchange of water for photosynthetic CO₂ (Franks & Farquhar, 1999).

The ability to push the limits of maximum productivity evolution through the evolution of increasing vein reticulation in angiosperms appears to be unambiguously advantageous (Brodribb *et al.*, 2007; Noblin *et al.*, 2008). Yet the developmental ability of the procambium to proliferate veins into several vein orders predates the evolution of angiosperm with high productivity and such a developmental ability has evolved in several other clades (Boyce, 2005; Feild & Balun, 2008). Such an evolutionary pattern raises the question 'Why is there an evolutionary constraint on the quantitative density of leaf veins?' Evidence of constraint becomes clear when considering the fact that none of the other evolutions of angiosperm-like venation systems in seed plants and ferns ever evolved high vein density over a long period of geologic time (i.e. Permian to recent) that experienced dramatic environmental (including low CO₂) and phylogenetic change (CK Boyce, TJ Brodribb, TS Feild, MA Zwieniecki, in review).

Before high vein density can be incorporated into the leaf, the veins must be narrow, whereas also providing high intrinsic conductive capacity to deliver water efficiently over a long,

highly branched pathway. These functional conditions apply because the leaf receives diminishing hydraulic returns from increasing the density of thick bundles of inefficient veins that displace mesophyll that otherwise could be used for photosynthetic carbon gain (Brodribb *et al.*, 2007; Noblin *et al.*, 2008). We suggest that angiosperms achieved a novel ability to produce such long, narrow, and high-efficiency veins by developing vessels in the primary xylem. Highly efficient vessels could facilitate a lowering of the cross-sectional investment of vein xylem area. The net result is that a given allocation of vascular tissue will ramify more extensively in the leaf whereas at the same time displacing a lower volume of photosynthetic mesophyll tissue. Unfortunately, it is not yet possible to evaluate the vessel hypothesis as an important key for high vein-density evolution because understanding the patterns of vessel evolution in basal angiosperm leaves remains to be documented.

Extant phylogeny also suggests that the evolution of increased leaf hydraulic capacity evolved first, with tolerance to drought evolving later. We can tentatively conclude this physiological pattern because several magnoliids, basal monocots, and basal eudicots have evolved high vein densities yet these species are shade-intolerant plants that occur in lowland tropical environments experiencing modest hydrodynamic gradients (Fig. 1). Future studies focusing on examples of basal eudicots (such as Buxaceae, Proteaceae, and Berberidaceae) and magnoliids (Canellaceae and Laurales) that contain radiations into drought-prone habitats would be helpful in discovering the traits that permitted complex vein reticulation to occur in xeric zones (Kohler & Bruckner, 1989; Jordan *et al.*, 2008).

Interestingly, water lily leaves appear to be exceptions to the rule that high vein density is a prerequisite to the evolution of high productivity. The floating leaves of Nymphaeales often have high photosynthetic rates and stomatal conductances to water vapor approaching $30 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and $600 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ (Fig. 2). Yet leaf vein densities in water lilies are considerably lower, ranging from 0.46 to 5.8 mm mm^{-2} , than the densities that would be expected (at least 16 mm mm^{-2} ; Brodribb *et al.*, 2007) to achieve such high gas exchange fluxes. To enable high CO_2 fixation ability, floating leaves of water lilies develop high stomatal densities on their adaxial surfaces and thick ($\sim 800 \mu\text{m}$) palisade systems with three to five tiers of cells (Kaul, 1976). Such expansive palisade systems signal that the mesophyll is densely packed with photosynthetic cells with high intrinsic biochemical capacities (Kaul, 1976; Smith *et al.*, 1997). Because of the largely shared diffusional pathway for CO_2 and water vapor, the low vein densities of Nymphaeales leaves suggest that large portions of the mesophyll should be under-supplied with water and therefore incapable of the high measured fluxes of gas exchange despite the presence of high stomatal density and thick palisade tissues (Brodribb *et al.*, 2007).

An option to increase the irrigation of the mesophyll in a way independent of veins is that other anatomical structures

deliver water very close to the sites of stomatal evaporation. In the context of their aquatic habitats, epidermal hypodermis are good candidate structures to fulfill such a nonvascular hydraulic system (Kaul, 1976; Lavid *et al.*, 2001). Hypodermis are $\sim 10 \mu\text{m}$ wide water- or mucilage-filled epidermal pores that are densely distributed (i.e. about 30% to 50% of the stomata density) only on the water immersed under surfaces of all leaves of water lily species. Although a direct role in leaf transpiration remains to be established, hypodermis have been demonstrated to transport xylem mobile ions from the aqueous environment (Lavid *et al.*, 2001). Much more experimental work would be useful for better understanding the sources and regulation of transpired water in floating Nymphaeales leaves. For example, application of tritiated water to hypodermis would be a useful experiment to establish if hypodermis act as gateway for water transport into the leaf mesophyll (Pedersen, 1993).

Nonetheless, the water lilies are the exceptions that support the hypothesis that increasing leaf vein density played a fundamental role in the evolution of increased productivity by angiosperms. Nymphaeales demonstrate that other requirements for high productivity, such as high CO_2 fixation biochemistry, palisade tiers specialized for high optical processing, and high stomatal density could be explored by the early angiosperm development toolkit as approximated by extant basal clades. However, only with the evolution of increased vein density, which is unavailable to extant basal clades, did it become possible to shift these high-productivity traits to the land (Smith *et al.*, 1997).

Wood hydraulic function and structure

Consistent with ancestral xerophobia of the angiosperm leaf, the xylem pipeline conducting water from roots to the leaves of basal angiosperms demonstrates ecophysiological incompatibilities with life outside of evermoist humid zones. Phylogenetic patterns from extant basal angiosperms imply that their common ancestor was woody (Feild *et al.*, 2004) and that the xylem operated with a low maximum stem hydraulic capacity on a sapwood area basis (K_S , an expression of xylem hydraulic efficiency relative to the amount of wood invested) of $0.61 \text{ kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$ (Table 1). Low capacities of the sapwood to conduct water are characteristic of most basal taxa across a range of life-forms and clades (Sperry *et al.*, 2007). Furthermore, the stem xylem is inferred to have lost half of its xylem hydraulic conductivity at a tension of -2.5 MPa (Table 1). These values for hydraulic capacities and vulnerability of the woody xylem to drought effectively confine these ancestral wood types to moist habitats where moisture remains predominantly high, and therefore the cost of carbon acquisition in the amount of water exchanged is low (Brodribb & Feild, 2000; Hacke *et al.*, 2006, 2007; Maherali *et al.*, 2006; Sperry *et al.*, 2007).

Structurally, why are the xylem systems of extant basal angiosperms hydraulically inefficient? Although woods of a



Fig. 2 The growth habit of *Amborella trichopoda* (Amborellaceae) illustrating the mechanical laxness of shoot biomechanical support in a vesselless angiosperm. (A) An overall view of the pendulous architecture of *A. trichopoda* in the understory. (B) Close-up of the basal branching pattern. Pithy water shoots that are overarch the base of the plant are iterated along the main leaning trunk. (C) Occasionally, plants also produce vine-like canes that ramify through the forest understory. A Plants of *A. trichopoda* were observed on Massif Aoupinie, 850 m, Province Nord, New Caledonia. Scale bars in each panel are 50 cm.

few basal taxa have tracheid-based water transport systems and therefore are expected to have low water transport capacity, most basal taxa possess vessels (Carlquist, 1975, 1982, 1984, 1999, 2001a). Xylem vessels have long been viewed as one of the decisive traits that catapulted the angiosperms to their current ecological dominance by greatly increasing xylem hydraulic capacity (Bond, 1989; Sperry, 2003). The evolution of vessels has been held as evolutionarily advantageous because vessels should permit much greater intrinsic hydraulic efficiency of the xylem because vessels are substantially wider and longer than tracheids, their evolutionary antecedents (Bailey, 1944; Carlquist, 1975; Doyle *et al.*, 1982; Doyle & Donoghue, 1986; Bond, 1989; Sperry, 2003). Higher hydraulic capacity of the xylem means that for a given amount of transpiring leaf area supported by the xylem, less carbon must

be used in the construction of a vascular pipeline and the same amount of transpiration can occur at smaller drop in Ψ from stem to leaf (Brodribb & Feild, 2000; Sperry, 2003).

Thus, measurements of wood hydraulic capacities, defined as xylem hydraulic flux divided by the cross-sectional area of xylem (K_S), of vessel-bearing basal angiosperms that were as low or even lower than vesselless angiosperms and conifers, was an unexpected result (Hacke *et al.*, 2007; Sperry *et al.*, 2007). Additionally perplexing, similar K_S occurred despite the fact that the diameters of vessels in basal taxa were greater than the diameters of vesselless angiosperm tracheids (vessels, range 30 to 90 μm , mean = 50 μm versus tracheids, range 9 to 27 μm , mean = 20 μm ; Sperry *et al.*, 2007). The general view of xylem conduit hydraulics posits that small increases in conduit diameter should provide much greater flow (Sperry,

2003). Indeed in vessels of derived eudicots, species with average vessel diameters equivalent to those of basal vessels transported water with far less resistance (Hacke *et al.*, 2006; Sperry *et al.*, 2007). Thus, a resistor or a set of resistors inside basal vessels was posited to have reduced the hydraulic gains that should have been produced by the development of wider conduits.

Considerable anatomical data indicate that the textbook dichotomy of how tracheids and vessels hydraulically function – vessels are hydraulically better than tracheids because of substantially longer and wider conduits – is extensively blurred in extant basal angiosperms (Carlquist, 1975, 2001a; Carlquist & Schneider, 2002). Extant basal angiosperms present a diverse spectrum of xylem anatomical types that range from the probable ancestral state of vessellessness to types with vessels bearing a varying range of small structural departures from tracheids. These tracheid-like vessels, hereafter called ‘basal vessels’, often partially retain their pit membranes between individual vessel elements making up a vessel, and they have primitive elongated ladder-like regions of pits at the ends of the vessel elements called scalariform perforation plates. Scalariform perforation plates, to varying degrees, resemble the inter-tracheid endwalls that occur in vesselless angiosperms (Carlquist, 1975). In contrast, most angiosperms have a single pore at the ends of connected vessel elements that nearly spans the width of the cell lumen. Such perforation plates are referred to as simple.

The anatomical blurring of the tracheid and vessel distinctions in most extant basal angiosperm lineages has been hypothesized as the structural basis for why their woods, despite having vessels, conduct water inefficiently relative to most derived angiosperm species that have been measured (Carlquist & Schneider, 2002; Sperry *et al.*, 2007). Indeed, the scalariform perforation plates of basal vessels were identified as major impediments to flow, accounting for nearly 60% of the total flow resistivity (Sperry *et al.*, 2007). The large resistance of scalariform perforation plates in basal vessels came as a surprise because previous estimates indicated that their flow obstruction effects were small (Schulte & Castle, 1993; Ellerby & Ennos, 1998). In contrast to previously modeled scalariform plates, however, the plates in the vessels of extant basal angiosperms are more prominent in a single vessel element in making up 30% to 60% of each element’s length (Carlquist, 2001a; Carlquist & Schneider, 2002). Also in their construction, the scalariform perforation plates of most basal angiosperms are more tracheid-like. The plates often consist of numerous and narrowly spaced bar-like pits (generally greater than 40 and up to 200 individual pit bars) that often retain partially degraded pit membranes (Carlquist, 2001a; Carlquist & Schneider, 2002). Besides scalariform perforation plates, additional resistors reducing the hydraulic capacities of basal angiosperm woods relative to eudicots with simple perforation plates include a low number of vessels per area of sapwood as well as shorter vessel lengths for a given conduit radius (Sperry *et al.*, 2007).

Finally, some evidence suggests that vessels of basal angiosperms with primitive scalariform perforation plates reduce the resistance of the xylem to drought. Species-mean cavitation pressures for basal taxa with vessels averaged -1.8 MPa, whereas vesselless angiosperms averaged -3.2 MPa (Hacke *et al.*, 2007; Sperry *et al.*, 2007). These differences in drought stress response were not associated with habitat differentiation as both functional groups generally occur together in everwet forests. Instead, the loss of safety from drought cavitation in taxa with basal vessels relative to vesselless woods was proposed to result from a functional constraint (Sperry *et al.*, 2007). Mechanistically, the initial opening of the pit membranes, which is the first event of vessel origination, increases endwall porosity between two cells. Although reducing hydraulic resistance between cells, the increased porosity of the pit membranes will lower the amount of pit membrane resistance that checks the expansion of tension-triggered embolisms through the pit membranes of one cell to another into the next following cavitation. Thus, the ease with which embolisms can spread will increase, resulting in a greater loss of xylem hydraulic capacity for a given tension (Sperry *et al.*, 2007). However, presumably soon after this developmental step, other pit membranes become less porous in order to delimit longer functional conduit lengths (Hacke *et al.*, 2007; Sperry *et al.*, 2007). During vessel developmental evolution, the amount of vessel element surface area occupied by pit membranes likely decreases, resulting in an increase in conduit resistance to cavitation (Hacke *et al.*, 2006, 2007; Sperry *et al.*, 2007).

Early evolution of vessels and wood function

The evolution of the angiosperm vessel has long been discussed as an instant success story, with the evolution of the bigger conduits of vessels reaping substantial savings in the water and carbon costs of transpiration (Carlquist, 1975; Bond, 1989; Sperry, 2003). Consequently, vessels have been portrayed as an evolutionary ratchet, escalating angiosperm ecological diversification by increasing hydraulic capacity and plasticity, growth rates, and drought tolerance (Carlquist, 1975; Doyle & Donoghue, 1986; Bond, 1989). Indeed, nearly all angiosperms have vessels, and those with the most derived vessels function with many of the highest productivities known.

Extant basal angiosperms paint a different picture of the early functional consequences of vessel evolution. First, the initial exploration of vessel morphospace and function did not increase overall wood hydraulic capacity. And perplexingly, experimentation with vessels may have reduced the resistance of the xylem to drought-induced cavitation in comparison to the ancestral tracheid-bearing state (Hacke *et al.*, 2007; Sperry *et al.*, 2007). Given these two functional consequences, why did vessels ever evolve in angiosperms?

Answers are still unclear for the initial advantage of vessels, but one untested idea is the ‘heteroxly hypothesis’. The hypothesis proposes that vessels permitted the xylem to diversify

in new directions of structural and functional heterogeneity (Feild & Arens, 2007; Sperry *et al.*, 2007). Motivating the hypothesis, woods of several species with basal vessels were found to be more hydraulically efficient on a *xylem conduit area basis*, defined as hydraulic flux normalized to the cross-section area of xylem conduits, than the tracheids of vesselless angiosperms, which approximate the ancestral state before vessel evolution (Sperry *et al.*, 2007). Increases in flow at the conduit scale by basal vessels, however, did not scale up to the entire stem cross-sectional level because basal vessels were sparse for a given surface area of wood. Nonetheless, in comparison to vesselless angiosperm woods, the low hydraulic capacities of angiosperm woods with basal vessels resulted from a smaller allocation of cross-sectional xylem surface area, and by extension volume, of wood devoted to hydraulic conduits (Sperry *et al.*, 2007).

A consequence of more-for-less space economy in the wood conveyed by vessels is that woods with primitive vessels may be freed to pack in new cellular types and functions and/or increase the allocation of existing cell types related to storage or radial transport as compared to vesselless wood. But importantly, the exploration of these new directions of structural and functional evolution does not entail a loss of xylem hydraulic capacity because the intrinsic efficiency of xylem conduits is increased. Maintenance of stable xylem vascular supply during the initial exploration of vessel morphospace is important because the ability of downstream leaf tissues to maintain photosynthesis with fewer water supplies is low in extant basal angiosperms (Table 2).

Another significant way that vessel evolution opens up more morphospace options in the xylem is that water conduction and mechanical support functions can be independently specialized upon by vessels and fibers, respectively, which are two types of cells that evolved from tracheids (Carlquist, 2001a). The tracheids of vesselless woods in species with a bifacial cambium, by contrast, multitask mechanical and hydraulic functions. Thus, tracheid-based vasculature must to some extent compromise hydraulic function to provide canopy support that results in a limited diversity in tracheary element shape (Carlquist, 1975, 2001a; Sperry, 2003). Although the phylogenetic diversification of xylem morphospace evolution remains to be quantified, the anatomies of extant basal angiosperms grossly indicate that the number of cell types in the conduit and ray systems as well as plasticity in xylem cell sizes and shapes were acquired gradually (Carlquist, 2001a, 2003; Carlquist & Schneider, 2002).

Alternatively, it could be argued that early fiber evolution, by offering new mechanical options, could function just as an effective catalyst as vessel evolution in opening the door to new vascular specializations. So why is there a bias toward viewing vessels over fibers as initial diversifiers of wood function (Carlquist, 1975; Doyle & Donoghue, 1986; Bond, 1989)? A hypothesis of vessels as motivators of xylem lability is supported by the observation that juvenile and many mature woods of Austrobaileyales and Chloranthaceae with vessels

produce large numbers of tracheids or tracheid-like fibers that probably conduct water (Carlquist, 1984, 1999, 2001a,b). These features appear to compromise biomechanical support (Carlquist, 2001a). Consistent with such a prediction, branching architectures of basal angiosperms having these anatomies exhibit a range of scandent patterns (Feild & Arens, 2005, 2007; Feild, 2008). Another difficulty with specializing fiber mechanics first is that without increases in intrinsic conduit efficiency, stem hydraulic capacity will decrease. Although plants could reduce the number of leaves or increase the amount of wood to support a given shoot transpiration rate and maintain a low range of Ψ_{leaf} deficits (Sperry, 2003), both allocation changes would reduce competitive ability under dense forest canopy shade, which some evidence supports as the ancestral habitat of angiosperms (Feild *et al.*, 2004; Feild & Arens, 2005, 2007; Feild, 2008).

The growth habit of *Amborella trichopoda* bears in a revealing way on the plausible direction of hydraulic-mechanical evolution in angiosperms. Although vesselless, *A. trichopoda* from seedling to adult has a pendently branched architecture (Fig. 2A,B). Also, *Amborella* is capable of producing vine-like canes (Fig. 2C). Given the basal phylogenetic position of *Amborella*, these architectural observations suggest the ancestral vesselless wood of angiosperms was skewed more toward hydraulics than mechanics as vessels evolved. In the future, a comparative analysis of correlated evolution vessel-fiber structure with wood hydraulics and biomechanics among basal angiosperms would be helpful for testing if during the initial steps of vessel-fiber co-evolution, optimization of mechanics lagged behind hydraulic efficiency.

The new developmental options associated with heteroxily may have been evolutionary advantage for early angiosperms in their ancestral habitats as understory plants adapted to damp, frequently disturbed environments (see Feild *et al.*, 2004). In these zones, plants are frequently damaged by falling canopy debris and the movements of errant animals (Bond & Midgely, 2001; Klimesova & Klimes, 2007). Also, the light-limited primary forest understory is fundamentally a 'light desert' for plant growth. Thus, injuries in the shade are especially serious in removing leaf and wood tissues built from hard-won CO₂ uptake or in exposing broken tissues to pathogen attack. We suggest that vessel evolution facilitated increases in the banking of carbohydrates and/or allowed internal replumbings of xylem tissues for radial resource transport to support sprouting buds that permit shoot reiteration following disturbance. Strategies of carbon storage are widespread among the stem and root vasculatures of extant basal clades (Carlquist, 2001b; Feild *et al.*, 2004; Feild, 2008). Also, as discussed above, we suggest that the initial evolution of vessels favored the evolution of scandent architectures. These architectures may have found utility in disturbed, shady microsites beneath the forest canopy by increasingly whole plant light harvesting efficiency. Also, these growth forms increase meristem redundancy across the forest floor, which

reduces the probability of an individual being killed by a single disturbance (Feild *et al.*, 2004; Feild & Arens, 2005, 2007).

The low drought tolerance of extant basal angiosperm xylem hydraulics indicates that evolution of the angiosperm vessel required wet environments (Sperry *et al.*, 2007). The requirement of a wet environment emerges because under high transpirational demand, the large flow resistance of most basal angiosperm woods imposes pressure drops in the xylem that would lethally desiccate the shoot. Although xylem drought tolerance may have decreased during the inception steps of vessel evolution that increase pit membrane porosity (Sperry *et al.*, 2007), the ease of refilling provided by frequent and strong root pressure in wet habitats likely offsets some of these constraints. Thus in wet environments, early experiments with vessels could take root and escape extinction by embolism.

Interestingly, the scalariform perforation plated vessels with a large number of pit bars persist rather deeply in extant angiosperm phylogeny. These primitive vessels often occur in low species diversity clades at the base of magnoliid and eudicot phylogenies (Carlquist, 2001a, 2003). Such a pattern suggests that the evolution of hydraulically efficient vessels bearing simple perforation plates was a gradual process. Interestingly, most members of these basal magnoliid and eudicot clades also appear to occur in everhumid environments (Fig. 1; Carlquist, 2001a). In future evolutionary analyses of the xylem traits possibly related to exporting vessels to a diverse range of flow capacities as well as environments, it would be useful to examine extant basal lines within major clades of angiosperms with wet-adapted ecologies as compared to close relatives that have radiated into dry zones. Similar to situation for leaves, the basal eudicot lineages of Buxaceae, Berberidaceae, and Proteaceae as well as the magnoliids Canellaceae and the Laurales would be ideal candidate lineages for such an analysis. A broad comparative ecophysiological analysis of coordinated evolution in vessel traits related to flow efficiency (i.e. conduit diameter and length distributions, perforation plate form, and vessel density) as well as drought tolerance to cavitation (i.e. area of the vessel elements occupied pits as well as pit membrane porosity) in these lineages would be informative (Sperry *et al.*, 2007).

Flower water use in relation to structure

Like the leaves and wood, the flowers of extant basal angiosperm lineages appear to require constant and generous supplies of water for function. Unlike in the previous sections, there has been little inquiry so far on the water balance of basal angiosperm flowers and how water balance affects reproductive function. Thus, many of the conclusions that we form in the succeeding discussion about the water economy of basal flowers and their evolutionary implications are speculative. However, we believe at the present time, the observations that we present provide a framework of testable hypotheses to guide future studies to understand the role of water in the evolution of early angiosperm flowering function.

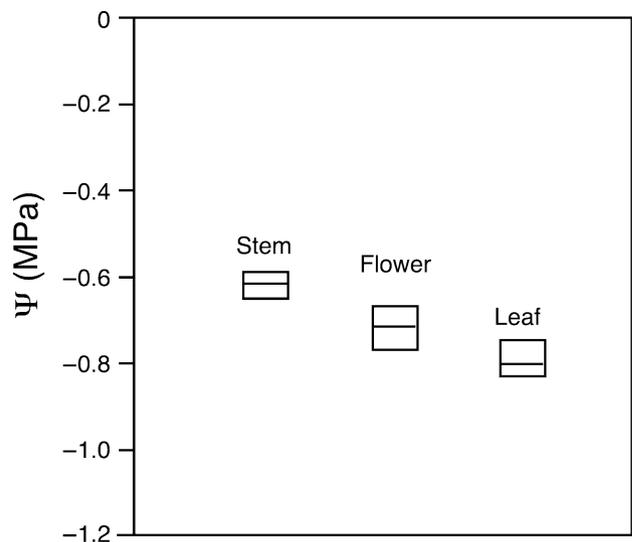


Fig. 3 Box plots of midday water potentials (Ψ) of stem tissues, leaves, and flowers of *Illicium anisatum* (Illiciaceae). The data demonstrate that Ψ of the flowers closely follows that of the stem xylem Ψ . Ψ Values were measured with a pressure chamber using accepted techniques. Stem xylem Ψ was approximated by measuring Ψ of leaves that were covered so that they did not transpire. These covered leaves are assumed to be in equilibrium with xylem Ψ . Each box plot represents three measurements of a plant organ type from three different plants. The center line denotes the average and the width of the box the standard deviation around the mean. Data were collected during June 2008 from plants raised outdoors in Knoxville, TN, USA.

New ecophysiological observations on flowers of two basal angiosperm species suggest that basal angiosperm flowers are hydrated mainly by the xylem (Fig. 3; TS Feild, DS Chatelet, TJ Brodrigg, unpublished observations, 2008). In contrast, all eudicot flowers studied to date for their water balance performance have been demonstrated to be supplied with water by living phloem tissues (Trolinder *et al.*, 1993; Chapotin *et al.*, 2003; De la Barrera & Nobel, 2004; Galen, 2005). For instance, the water potentials of mature flowers (Ψ_{flower}) of *Illicium anisatum* (Illiciaceae) and the magnoliid *Magnolia grandiflora* (Magnoliaceae) tracked diurnal decreases in stem water potential (Ψ_{stem}) during transpiring conditions (Fig. 3; TS Feild, DS Chatelet, TJ Brodrigg, unpublished observations, 2008). In addition, Ψ_{stem} to Ψ_{flower} gradients were downhill for water flow into the flowers because Ψ_{flower} values were more negative than those of Ψ_{stem} in both species. These gradients were unaffected by girdling of the stem bark, which severs possible water supplies coming from the phloem (TS Feild, DS Chatelet, TJ Brodrigg, unpublished observations, 2008). Consequently, it appears that the flowers of these basal angiosperm species function in close association with xylem water status (Chapotin *et al.*, 2002). Although much work needs to be conducted on how the vascularization of basal angiosperm flowers relates to water transport, anatomical studies indicated that xylem tissues ramify extensively near the stigmatic surface as well as along the pollen tube pathway and ovule cavity in *Amborella*, *Illicium*, and *Trimenia* (Robertson & Tucker, 1979; Endress & Sampson, 1983; Williams, 2009).

Such tissues appear to be well positioned to maintain the hydration state of the flower when Ψ values of the rest of the plant are high.

Observations of flower exudation phenomena in extant basal angiosperms also provide evidence for the close connections of flowers to the xylem. For example, floral bracts in inflorescences of several Chloranthaceae become studded with water droplets produced by root pressure-driven water flow that flows through the xylem from roots to the flower (Feild *et al.*, 2005). Composition of the bract liquid is not known, but water content (WC) appears to be high because droplets are not viscous like the sugar- and protein-rich pollination droplets of many gymnosperms (Feild *et al.*, 2005; Mugnani *et al.*, 2007). Interestingly, the tips of floral bracts of some basal angiosperms structurally parallel chloranthoid teeth (von Balthazar & Endress, 1999; Buzgo *et al.*, 2004; Feild *et al.*, 2005). The watery, low-sugar content stigmatic pools formed in many water lily flowers, such as *Nymphaea*, *Nuphar*, and *Ondinea*, may be other examples of xylem flow-dependent secretions (Schneider & Chaney, 1981; Schneider, 1983). However, other floral secretions in basal taxa likely occur via nonxylem pathways. For example, *Austrobaileya scandens* (Austrobaileyaceae) vines that lack root pressure exude whitish fluid from floral staminodes (Endress, 1984). Phloem-originating water supply may be involved in this process. More observations on the diverse exudation phenomena in basal angiosperms would be highly informative (Endress, 2001, 2008).

The major physiological consequence of a flower water economy predominantly dependent upon the stem xylem is that flowers will compete with leaves that also attach to the stem xylem. Versus leaves, some observations indicate that flowers of basal taxa can be the losers in the tug-of-war for water when xylem tensions become strained. On whole plants of *Illicium* (*I. anisatum* and *I. parviflorum*), *Chloranthus japonicus*, and *Magnolia grandiflora* that are challenged with soil drying, flowers visibly wilt sooner than adjacent leaves (TS Feild, unpublished observations, 2008). Once the wilting threshold has been reached, flowers of some basal angiosperm species did not recover. For example, species such as *Illicium parviflorum* and *Kadsura longipendunculata* often abscise the entire flower following a single wilting event (TS Feild, unpublished observations, 2008). These observations suggest that flowers have even higher Ψ of TLP than leaves. Recent measurements on tepals of *M. grandiflora* support this conclusion at least in *M. grandiflora* with the TLP of tepals occurring at -0.7 MPa whereas the TLP was -2.2 MPa in leaves (TS Feild, DS Chatelet, TJ Brodribb, unpublished observations, 2008).

It is interesting to note that most basal angiosperms are profligate sprouters with long-lived belowground storage organs that include rhizomes and ligno-tubers (Feild *et al.*, 2004; Feild & Arens, 2005). Thus, if flowering fails to set seed resulting from drought, many taxa would seem to be able to persist in a preferred site by sprouting until another bout of flowering. Also, sprouting may provide opportunities for

failsafe vegetative reproduction and population spread if the sprout ramets become severed from the original genet during disturbance (Bond & Midgley, 2001; Feild *et al.*, 2004; Feild, 2008). A disadvantage of the sprouting strategy is that storage comes at a cost of decreased extension growth to reach high light intensities above the forest floor.

Turning to the interior water relations of basal angiosperm flowers, the mode of angiospermy in basal angiosperms also signals xerophobia. Phylogenetic analyses on extant basal angiosperm flowers indicated that the ancestral form of the angiosperm carpel was bottle-shaped (ascidiate) and possessed unfused stigmatic canal(s) forming intercellular spaces. These spaces were also filled with extracellular gel-like secretions extruded from the cells of the carpel from the from the stigmatic surface and all the way to the ovules (Endress & Igersheim, 2000a,b; Endress, 2001; Williams, 2008, 2009). Germinating pollen tubes of most basal taxa appear to grow exclusively or for the most part through these secretions (Williams, 2009). What molecules compose these gels remains to be fully characterized. However, mucilages, such as pectins, arabinogalactans, and arabinogalactans complexed to proteins, are abundant and phylogenetically widespread constituents of these secretions (Endress & Sampson, 1983; Bernhardt *et al.*, 2003; Thien *et al.*, 2003; Lyew *et al.*, 2007; Sage *et al.*, 2009).

Although some of these molecules participate in signaling cascades related to reproduction and cell adhesion biochemistry (Bernhardt *et al.*, 2003; Sage *et al.*, 2009), the occurrence of mucilage suggests that high WC and Ψ values are necessary for essential carpel functions to operate in basal taxa with ascidiate carpels (Nobel *et al.*, 1992; Chapotin *et al.*, 2003). Indeed, pure mucilage functions as a hydrogel and can hold nearly 100% of its dry mass in water (Guinel & McCully, 1986; Nobel *et al.*, 1992). Although mucilages have high WC, mucilage-bound water is rapidly depleted at slightly low Ψ values around -0.3 MPa (Guinel & McCully, 1986; Nobel *et al.*, 1992). Thus, water can readily move passively from extracellular mucilages into the walls of nearby metabolically active cells. Mucilage can therefore provide a well-hydrated setting for the water relations and biochemistry of pollen tube growth, stigmatic self-incompatibility, and ovule development, all of which represent processes that are sensitive to drought (Galen, 2005). Because mucilage so readily exchanges water, it can, however, only weakly buffer against drought stress (Nobel *et al.*, 1992). Once the mucilage is dehydrated, mucilage-rich regions or those that are structurally in-filled by mucilage, such as the pollen tube pathway, may possibly lose functionality when mucilages become dehydrated and retreat from intercellular spaces (Guinel & McCully, 1986). Future cryogenic scanning electron microscopic images of desiccating flowers would be informative for observing how flowers respond structurally to drought. How flowers of basal angiosperms, which can experience significant tensions during the day (Fig. 3), may avoid complete mucilage desiccation remains unclear. Alternatively, the functions of the flower taking in place in association

with mucilage may be permitted during environmental conditions of high whole plant Ψ , such as during rain and cloud forest mist, when root pressure occurs, or during the night.

Early evolution of the flower

Flowers are the seats of mate selection and the development of a novel sexually formed polyploid endosperm. Each of these innovations resulted in reproductive diversification on a large scale that created new trophic cascades as pollinating and dispersing biotas exploited new flower resources. Thus, flowers have long formed the major conceptual basis for numerous hypotheses on the origin and early ecological success of angiosperms (Stebbins, 1974; Regal, 1977; Gottsburger, 1988; Taylor & Hickey, 1996; Gorelick, 2001; Bateman *et al.*, 2006; Friis *et al.*, 2006; Crepet, 2008; Williams, 2008, 2009; Soltis *et al.*, 2009). Below we explore how the water balance of early angiosperm flowers, as inferred from living basal lineages, and in relation to the water relations of the rest of the plant may have influenced some major directions of early flower evolution. Although it is clear that interactions with pollinator vectors have played important roles in the early diversification of flower form (Thien *et al.*, 2000, 2009; Williams, 2008) and much more comparative work on extant basal angiosperm flowers is needed to understand their water-use functions, our working hypothesis for future comparative research is that functioning of early angiosperm flowers was significantly influenced by the water-use of the rest of the plant because early flowers were intrinsically drought intolerant and they received water from a drought-feeble transpiration stream.

The open flowers of basal angiosperms are generally small in cross-sectional size (Endress, 2001, 2008; Endress & Doyle, 2009). Besides minimizing the investment in reproduction, if flowers are connected to the xylem, then there several other good ecophysiological reasons for flowers to be small in drought-intolerant basal angiosperms. As compared to larger flowers and all else being equal, smaller flowers will have shorter hydraulic path lengths between the vegetative xylem that supplies water to especially drought-sensitive regions of the flower such as the stigmatic surfaces, pollen tube pathways, and ovules (Galen, 2005). Also, small flowers will transpire less because of small perianth surface areas. Less evaporative surface area should decrease the development of water stress gradients inside the flower during exposure to the sun and dry winds. Thus, small flowers of basal taxa will experience minimal drops in Ψ on top of those imposed by the vegetative xylem. Minimizing the pressure drops associated with hydraulic transport from the vegetative to flower tissues is especially important because if the flowers are connected to the xylem. To attract water from the transpiration stream, flowers will have to function at a more negative Ψ than the stem xylem (Fig. 3). Such functioning puts flowers at risk of drought-induced abortion (Galen, 2005). Therefore, one interpretation for the occurrence of small flowers at the base of angiosperm phylogeny is that avoidance of drought boxed in maximum flower size.

There are, however, important exceptions to the small-flowered pattern among basal angiosperm lineages (Endress, 2001; Schneider *et al.*, 2003; Endress & Doyle, 2009). For example, floating flowers of some basal angiosperm aquatics can be gigantic, at up to 20 cm across (Schneider *et al.*, 2003). However, other flowers of basal angiosperm aquatics are much smaller, such as the floating flowers of *Cabomba* and *Ondinea* (Schneider *et al.*, 2003) and underwater flowers of moss-like *Trithuria* plants ~2 mm, Endress & Doyle, 2009; but note some of these species exhibit tiny flowers that protrude somewhat above the waterline) and the highly reduced plant *Ceratophyllum* (at 3 mm).

The giant aerial water lily flowers seem to be exceptions that support the hypothesis that water balance can influence flower size evolution in extant terrestrial basal angiosperms. Because these flowers are connected to a vascular system with freely accessible water and they have specialized hydropotes that may supply water from the waterline (see previous discussion), we suggest that the aquatic zone may have relaxed xylem hydraulic constraints and permitted these species to evolve large perianth surface areas that lose large amounts of water at little hydraulic cost. Alternatively, the evolution of increased in flower size in the Nymphaeales may be a developmental byproduct associated with the evolution of giant lily pads in the species bearing the largest flowers such as *Euryale* and *Victoria*. Or, it may be a specialization toward beetle pollinations, which are most readily accommodated in large flowers (Thien *et al.*, 2009). Sorting out developmental cause and effect for flower size evolution in the water lilies is currently difficult to discern and requires future comparative physiological studies.

The flowers of the tropical cloud forest liana *Austrobaileya scandens* (Austrobaileyaceae), however, are a genuine exception (Endress, 2001). At 4 cm across, flowers of *A. scandens* are the largest of all extant terrestrial basal most angiosperm species. Flowers of *A. scandens* are also often borne high in the forest subcanopy where they should experience significant evaporative demand and possibly steep negative Ψ gradients imposed by the long hydraulic path from the flower to the soil. Yet *Austrobaileya*'s xylem and internal micro-hydraulics of the flower appear to be as xerophobic as other basal species (Endress, 2001; Williams, 2008; Table 1). At least from a water balance perspective, it remains unclear how *Austrobaileya* flowers are able to regulate their water use and become relatively large in air. One possibility, given their unusually waxy tepals, is that *Austrobaileya* flowers have evolved to simply resist perianth transpiration up in the cloud forest subcanopy. Another possibility is that the hydraulic efficiency of water transport to the flower has increased and therefore raised up the water stress ceiling on flower size. Such an explanation may apply to the large flowers of the magnoliid species *Magnolia grandiflora*, which has evolved a considerably larger Huber value, defined as the amount of xylem present at the pedicle base relative to the perianth surface area as compared to flowers hydrated by the phloem (TS Feild, DS Chatelet, TJ Brodribb,

unpublished observations, 2008). High Huber value supporting the flower perianth seems to be necessary for the evolution of large flowers in *M. grandiflora* because the tepals lose substantial amounts of water (TS Feild, DS Chatelet, TJ Brodribb, unpublished observations, 2008). A disadvantage of increasing xylem allocation, however, is that the carbon costs of a soon-to-be discarded perianth will be considerably increased due to expensive lignified tissues.

The hypothesis that early angiosperm flowers are xerophobic suggests that the maintenance of flower water balance represents an additional process that will need to be integrated with other more appreciated roles of flowers that have been proposed to have colored the selective regime responsible for evolutionary development of flower origin and the origin of the angiosperm's distinctive ability to reproduce efficiently and rapidly (Stebbins, 1976; Williams, 2008, 2009). Nearly all of the hallmarks of angiosperm reproduction, including closed carpels, miniaturization of male and female gametophytes, double fertilization, sexually formed polyploidy endosperm, and an exceptionally short pollination-to-fertilization interval have long been proposed to have evolved under selection for faster reproduction in association with environmentally marginal habitats, most often conceived as dry dynamic zones (Stebbins, 1965, 1974, 1976; Axelrod, 1972). However, the habitat distribution and water balance of ecophysiology of extant basal angiosperms suggest that the evolutionary beginnings of these innovations trace back to wet environmental conditions. Importantly, flowers of basal angiosperms today in wet zones (including basal water plants) display phylogenetic lability in size, color, the number and identity of perianth parts, phyllotaxis, and embryology (Endress, 2001, 2008; Schneider *et al.*, 2003; Zanis *et al.*, 2003; Friedman, 2006, 2008; Williams, 2008; Endress & Doyle, 2009; Soltis *et al.*, 2009). Thus, the extant diversity of flowers in wet-adapted basal angiosperms suggests that wet habitats can also represent dynamic habitats fostering reproductive innovation (Endress, 2001, 2008; Friedman, 2006, 2008; Endress & Doyle, 2009).

On one hand, wet terrestrial habitats may provide favorable habitats for evolutionary experimentation in flower form and function because such habitats decrease the intrinsic water costs of flowers and the feedbacks of a weak vegetative drought tolerance on reproductive function during drought stress providing that flowers remain small. On the other hand, wet terrestrial habitats offer dynamic environmental circumstances that potentially provided an important selective context for new efficiencies in reproductive function that were later co-opted for much more rapid reproduction and flowering in drier habitats (Williams, 2008). Wet habitats may favor evolutionary steps toward increasing reproduction rate because like other plant organs exposed to prolonged damp conditions, flowers are in race against time to evade pathogen invasion and rot. Indeed, bacteria swarm on the moist and receptive stigmatic surfaces of *Amborella trichopoda* flowers in the field (Williams, 2009).

Also, speeding up reproductive rate would be important to angiosperms in their reconstructed ancestral niche as colonizers of disturbed micro-sites in the forest understory (Feild *et al.*, 2003a, 2004; Feild & Arens, 2005, 2007; Feild, 2008). Increased rates of seed production, which are influenced by speeding up flowering and fertilization rates, increase the chances that seeds will find ephemeral sites that offer breaks in canopy shade and soil space opened up by forest understory disturbance.

Alternatively or in addition to pressures surrounding the circumventing of pathogen attack and colonizing disturbance opportunities, the avoidance of drought even in everwet habitats can still be a potent selective pressure for increase in reproductive rate, especially because flowers are attached to vegetative tissues prone to drought dysfunction. Extant basal most angiosperm clades suggest that the drought events that could influence the viability of evolutionary experiments in reproductive innovation are rescaled from those envisioned previously. Rather than avoiding six months without rain such as in the dry subtropical chaparral (Stebbins, 1974), inopportune sunflecks, dry winds, and forest gaps are evaporative challenges that can stress basal angiosperm flowers during anthesis and select for the evolution of rapid reproduction to complete anthesis to avoid desiccation stress.

Understanding how the water relations function in extant basal angiosperms points to another new plausible reproductive key innovation of early diversification. We hypothesize that breaking free of the stem xylem in favor of the phloem for hydration, which is seen in all of the derived eudicots studied so far, represents a major evolutionary transition of angiosperm reproduction (Trolinder *et al.*, 1993; Chapotin *et al.*, 2003; De la Barrera & Nobel, 2004). In these eudicots, the water needs of flowers are met by active loading of solutes into the vegetative phloem that generates a favorable hydrostatic pressure gradient toward the flower as these solutes are unloaded (Trolinder *et al.*, 1993; Chapotin *et al.*, 2003; De la Barrera & Nobel, 2004). By disconnecting most, if not all, of the stem xylem from the flower, the flowers gain the advantage that Ψ can be stably maintained at high level diurnally throughout anthesis and perhaps even during severe drought stress (Trolinder *et al.*, 1993). Indeed, Ψ values of whole flowers or petals that are phloem hydrated are often three to five times more positive than Ψ values of transpiring leaves and tissues throughout the day (Chapotin *et al.*, 2003; De la Barrera & Nobel, 2004). Thus, reproductive functions in the eudicots appear to function under relatively homeostatic water relations through receipt of water from the phloem. Increasingly the decoupling vegetative and reproductive water relations may also increase the exportability of reproductive innovations that evolved earlier in the wetter environments to drier conditions, such as under hot daytime conditions and less wet habitats. Furthermore, rerouting of flower water supply toward the phloem may enable new evolutionary directions in vegetative drought tolerance to be reproductively viable. This is because

the lower xylem tensions accommodated by new drought-tolerant traits would not conflict with the drought sensitivity of reproduction biochemistry (Galen, 2005).

The evolution of a more active control over water supply to flowers by the phloem, however, may create a physiological difficulty. The flower must regulate where the solutes (primarily sugars) end up, which occurs once water exits the sieve tubes. Otherwise, flowers will face a new drought problem – osmotic shock. One solution to avoid solute overload is to release these sugars as well as other products derived from phloem transport to the external environment as nectar (De la Barrera & Nobel, 2004). Such sugar dumping, however, results in an evolutionary windfall on the ecological stage. Angiosperm nectar represents a resource that catalyzed new evolutionary waves of pollinator fidelity and diversity (Grimaldi, 1999; Crepet, 2008). Curiously, guttation-bleed valves on floral bracts of several Chloranthaceae and *Amborella* appear to be way stations in the evolution of nectar secretion (von Balthazar & Endress, 1999; Buzgo *et al.*, 2004; Feild *et al.*, 2005). Although these structures have other functions related to root pressure (see previous discussion), all that would be involved to transform these structures into nectaries would be extensions of the phloem into the apical regions where xylem conduits irrigate the epithem (von Balthazar & Endress, 1999; Feild *et al.*, 2005), and the development of nectariferous tissues in the epithem in order to exude nectar during daytime transpiring conditions.

IS EXTANT BASAL ANGIOSPERM XEROPHOBIA A MESOZOIC ECOPHYSIOLOGY? A PRELIMINARY LOOK AT WHAT THE FOSSILS SAY

Clarity on the basal phylogenetic divergences in extant angiosperm molecular phylogeny and the increasingly detailed studies of the comparative structure and development of extant basal angiosperm lineages have redrawn our imagery of the earliest flowering plants (Doyle & Endress, 2000; Doyle, 2001, 2008; Feild *et al.*, 2004; Friedman, 2006, 2008; Endress & Doyle, 2009). However, the extant-based ancestral reconstruction program, including the approach featured here, has a possible Achilles' heel – ancestors phylogenetically deduced from living plants may have never existed.

Mismatches between the ecologies of early diverging clades and early angiosperms are conceivable in many ways (Crisp & Cook, 2005; Feild & Arens, 2005, 2007). These ways include (i) the extinction of clades that are more basal, which had with different ecologies from the surviving sample of basal lineages, (ii) the modification of ancestral Mesozoic features present in the ancestors within these basal lineages through adaptation to global-local environmental change or in response to associations with new organisms during the long evolutionary history of basal lineages (Stebbins, 1974; Sun *et al.*, 2002; Endress & Doyle, 2009). Since the Early Cretaceous, global climate warmed and cooled, CO₂ dropped, major herbivore lineages

became extinct and others diversified, pollinators and dispersers have come and gone, an asteroid fell and reworked communities, the continents shifted and sea levels rose and fell, and plant communities became predominately composed of angiosperms. What confidence then is there that extant basal angiosperms represent appropriate ecophysiological models for ancient angiosperms? Clearly, the fossil record will be the testing ground for addressing such a question, providing that fossils can be ecophysiologicaly dimensionalized.

Previous work indicated that the morphologies of extant basal angiosperm leaves, pollen, seeds, and flowers correspond well with those of some of the earliest known Cretaceous angiosperms (Upchurch, 1984a,b; Doyle & Endress, 2000; Doyle, 2001, 2005; Feild *et al.*, 2004; Feild & Arens, 2005, 2007; Friis *et al.*, 2006). Such similarity of fossil features with candidate extant relatives increases confidence in the phylogenetic relevance of extant basal lineages to the Early Cretaceous. When fossils have been assayed for function, some encouraging results have emerged. For example, despite extensive geographic extinction and recent speciation with the clade, an ancient 120-Ma long retention of wind pollination can be recognized within the *Hedyosmum* lineage based on fossil pollen structure and perianth form of related fossils (Friis *et al.*, 1999, 2000; Eklund *et al.*, 2004). But an analysis of the water balance traits of early angiosperm fossils, in relation to those of extant basal angiosperms lineages, has yet to be conducted. Such a consideration of early angiosperm paleoecology is essential as most previous ecological and some paleoenvironmental interpretations of the earliest known fossil angiosperms clash with the ancestral traits reconstructed from extant basal lineages (Doyle & Hickey, 1976; Doyle *et al.*, 1977, 1982; Hickey & Doyle, 1977; Retallack & Dilcher, 1981, 1986; Taylor & Hickey, 1996; Wing & Boucher, 1998).

Irrespective of whether living basal lineages are good or bad early angiosperm models, their water-demanding ecophysiologicals make explicit predictions of the ecomorphic make-up and paleoenvironmental contexts we should see in the oldest fossil angiosperms if the xerophobia hypothesis holds. Below, we present a preliminary examination of the Early Cretaceous records of angiosperm leaf, flower, wood, and pollen fossils for the specific ecomorphic traits as well as environmental contexts predicted from living basal angiosperms to look for early angiosperm xerophobia.

Leaf fossils

Although the earliest known angiosperm fossil leaves are clearly not the oldest fossilized angiosperms (see succeeding discussion), an important stratigraphic sequence that has inspired many hypotheses on early angiosperm ecophysiology is the Potomac Group from the Atlantic Coastal Plain in the United States (Doyle & Hickey, 1976; Hickey & Doyle, 1977; Retallack & Dilcher, 1981, 1986; Taylor & Hickey, 1996). The sequence preserves angiosperm leaves with venation

details as well as associated and dispersed cuticles in sediments that span a critically important timeframe for early angiosperm diversification. For example, the oldest sediments, referred to as Zone I (Brenner, 1963) and now dated as Aptian to early Albian, preserve angiosperms ~10 Ma before eudicots were represented by more than a few forms in the mid-paleolatitudes of Southern Laurasia (Doyle & Robbins, 1977; Hickey & Doyle, 1977; Upchurch, 1984a,b; Hochuli *et al.*, 2006). In the discussion below, we focus on the form and possible paleoecophysiologicals of the oldest Potomac Group leaves. Previous studies indicated that this fossil sequence contains species likely to be related to extant basal angiosperm lineages (Upchurch, 1984a,b; Doyle, 2001). Although additional lines of paleoecophysiological evidence need to be drawn from other Early Cretaceous angiosperm leaf assemblages (Vakhrameev, 1991; Cuneo & Gandolfo, 2005), details on the ecomorphology of these fossil leaves are not available from the literature.

The venation systems of the Zone I species mostly share a distinctive disorganized look of irregularly defined vein orders, variably shaped intercostal areas, and relatively large asymmetrical minor vein areoles. Based on these qualities, venations of Zone I leaves were described as 'first rank' patterns (Doyle & Hickey, 1976; Hickey & Doyle, 1977). Leaf rank, which varies from one to four, is a semi-quantitative metric to describe vein organization. Leaf rank number refers to the highest vein order in which veins tend to develop regular courses and delimit areas of relatively consistent size and shape. Extant basal angiosperms examined so far with first-rank venation organization have low vein densities (DS Chatelet, PJ Hudson, & TS Feild, unpublished data 2008). Consequently, first-rank venation may indicate resistive pathways of water transport and therefore low water and CO₂ gas exchange rates (Brodribb *et al.*, 2007).

Also, a few Zone I fossils bear chloranthoid glandular teeth on the leaf margin (see Doyle & Hickey, 1976; Hickey & Doyle, 1977; Upchurch, 1984a,b). As supported by extant basal taxa, chloranthoid teeth indicate frequent generation of root pressure and guttation (Feild *et al.*, 2005; Feild & Arens, 2007). By extension, the presence of chloranthoid teeth on fossil leaves indicates that at least some Early Cretaceous angiosperms occurred in frequently wet, well-drained rooting environments as well as humid conditions.

Independent evidence for wet habitats of early angiosperm leaves comes from paleoenvironmental contexts. At the regional scale, palynomorph assemblages, climatic-sensitive sediments, and paleoclimatic models indicate humid, high rainfall, and subtropical climates during Aptian times of the Potomac Group (Doyle & Hickey, 1976; Hickey & Doyle, 1977; Beerling & Woodward, 2001). At the local scale, the coarse-grained character and moderate carbon content of the sediments containing Zone I angiosperm leaves suggest that these leaves were deposited in or near active riparian channels and not anoxic back swamp zones in lowlands (Hickey & Doyle, 1977). Close proximity to water suggests ample hydra-

tion of plant roots, frequent high-humidity conditions, and therefore frequent opportunities for root pressure. However, it is unclear if the early angiosperm leaves deposited in these sediments were from plants growing in sunny-exposed point bars or if they came from understory plants occurring in forests along river channel that became covered by crevasse-splay deposits, or both (Feild *et al.*, 2004). Also, given the rarity, the mostly small sizes (<3 cm long; but note *Ficophyllum* leaves were possibly 20 cm long; Doyle & Hickey, 1976; Hickey & Doyle, 1977), and the fluvial ties of nearly all Zone I angiosperm leaves, long-distance wash in from other types of wet, extra-basinal regions is a possibility that has not been ruled out.

Although there are emerging indications of ecophysiological overlap among the earliest angiosperm fossil leaves and extant basal lines, a few morphological features of Zone I leaves do not have an extant basal analog. For example and similar to some flower fossils (Friis *et al.*, 1999, 2000; von Balthazar *et al.*, 2008), Zone I leaf cuticle fragments of taxa almost certainly related to extant basal grade angiosperms combine characters on a single leaf that are today retained in separate lineages (Upchurch, 1984a,b). This is to be expected if these taxa are stem lineages of extant lineages (Doyle, 2001). However, whether these nonextant character combinations in early angiosperm leaf cuticles generated nonextant ecophysiologicals remains unclear. A broad comparative analysis of extant basal angiosperm leaf ecomorphology, focusing on vein and stomatal traits, in relation to water use and habitats would allow for understanding the implications that different character associations have on the inference of water balance regulation of early angiosperm fossil leaves.

Flower and wood fossils

Numerous angiosperm fossil flowers discovered during the Early Cretaceous (reviewed in Friis *et al.*, 1999, 2000, 2006) reveal some broad similarities to those of some extant basal angiosperm lines. Although fossil flowers have not been viewed as paleohabitat indicators, the morphologies of some of the earliest known angiosperm flowers are broadly consistent with the morphological predictions of xerophobia. For example, Aptian and early Albian flowers are small in size and probably bore small perianths that would be predicted to lose small amounts of water to the environment (Friis *et al.*, 1999, 2000, 2006; von Balthazar *et al.*, 2008; Endress & Doyle, 2009). Also, the carpels of many of these ancient most angiosperm flowers are suggested to be ascidiate (Endress & Doyle, 2009). Analogizing these carpels to extant basal angiosperms may indicate the presence of internal hydrating mucilage, which may signal flower intolerance to drought. Future X-ray tomographic studies of flowers would be useful in diagnosing their possible internal hydraulics and therefore associations with wet habitats (Friis *et al.*, 2007).

Also, anatomical measurements of the cross-sectional xylem area at the flower pedicel or the tepal scars could provide clues

to discern if flowers were connected to the xylem, and may thus possibly be xerophobic, by quantifying the amount of xylem developed in a fossil flower. This is because extant basal angiosperms with xylem-hydrated flowers have much greater amounts of xylem for a given perianth surface area as compared to phloem-hydrated flowers (Feild & Chatelet, in review). Another important approach to understand the hydrological envelopes of early angiosperm flowers would be to conduct analyses on the ecomorphic characters of the fragmentary remains of angiosperm leaf tissues that are often reportedly deposited with the sediments containing fossil flowers. These studies could define the range of vegetative water use physiologies associated with a given sample of flower diversity (Friis *et al.*, 1999). These fossils may be particularly important because they are most likely fossilized *in situ*. However, these sediments are much younger than the earliest angiosperm fossil pollen grains known (see succeeding discussion).

The available functional anatomical details on the wood structure and function of the earliest angiosperms remain sketchy, primarily because fossil angiosperm woods are rare during the Early Cretaceous (Wheeler & Baas, 1991; Wing & Boucher, 1998; Philippe *et al.*, 2008). Also, all known specimens are much too geologically young to represent the earliest angiosperms (Wheeler & Baas, 1991; Wing & Boucher, 1998; Philippe *et al.*, 2008). Also, no wood fossils discovered so far can be linked to any extant basal angiosperm clade. Thus before any definitive conclusions can be made about the hydraulic function of early angiosperm wood, much more work is needed on the structure of angiosperm fossil woods from the Early Cretaceous.

Toward such a goal and given that extant phylogeny suggests that angiosperms were most likely ancestrally woody (Doyle & Endress, 2000; Feild *et al.*, 2004), developing a taphonomic understanding of possibly why early angiosperm woods are so rare would be informative. For example, decomposition studies made on extant basal angiosperm woods conducted in different depositional sediments could inform the search images and expectations of the specific deposition environments to discover putative new fossil angiosperm wood fossils in the future. Indeed, wood fossils of other non-angiosperms are abundant in Late Jurassic to Early Cretaceous, indicating there is not a taphonomic mega-bias against wood fossilization during such a time frame (Philippe *et al.*, 2008). If more evidence of angiosperm fossil woods can be discovered, our understanding of conduit hydraulics has now reached the stage that flows can be modeled from fossil xylem anatomy to calculate stem xylem hydraulic capacities and, to some extent, the tolerance of the xylem to drought-cavitation (Sperry *et al.*, 2007; Wilson *et al.*, 2008).

Pollen fossils

Fossil pollen grains represent the oldest accepted traces of the angiosperm lineage. In fact, the oldest pollen fossils

from the Valanginian-Barremian of the Early Cretaceous precede the oldest known angiosperm leaves and flowers by 20 Ma and wood by at least 25 Ma (Wheeler & Baas, 1991; Brenner, 1996; Doyle, 2001; Philippe *et al.*, 2008). Thus, fossil pollen is the only direct window on the most ancient phases of early angiosperm evolution. The first angiosperm pollen fossils are represented by monosulcates with morphologically diverse external sculpturing (Doyle & Robbins, 1977; Doyle *et al.*, 1977, 1982; Hickey & Doyle, 1977; Hughes, 1994; Brenner, 1996). However, these fossils are rare, occurring at less than 1% of the total abundance nonangiosperm palynomorphs (Heimhofer *et al.*, 2005, 2008; Hochuli *et al.*, 2006).

Systematic studies of these fossils would indicate wet demanding ecophysiologicals for the parent plants. For example, some ancient grains assigned to *Clavatipollenites* are likely related to Chloranthaceae (Walker & Walker, 1984; Pedersen *et al.*, 1991; Doyle, 1999; Friis *et al.*, 1999, 2000; Eklund *et al.*, 2004). Another chloranth pollen type, *Asteropollis*, occurs first in Aptian sediments. *Asteropollis* appears similar to wind-dispersed pollen of *Hedyosmum*, and *Hedyosmum*-like fossil flowers with *Asteropollis* grains attached are known from Aptian sediments (Friis *et al.*, 1999, 2000). Finally, rare pollen grains resembling *Amborella* have been found in Hauterivian sediments of England (Hughes, 1994; Doyle, 2001, 2005). However, it is important to note that several other fossil pollen types cannot be assigned to extant lineages (Doyle & Robbins, 1977; Doyle *et al.*, 1977, 1982; Hughes, 1994; Heimhofer *et al.*, 2005; Hochuli *et al.*, 2006; Zavada, 2007). In addition, many extant basal angiosperms lack Early Cretaceous fossil pollen records.

Independent lines of evidence for wet climates for some of these fossils came from diverse sources. Climatic-sensitive lithological indicators, atmospheric modeling efforts, dinosaur fossils, and the broader community context of co-occurring palynomorphs show that some mesic climates existed amidst the continental arid lands that dominated much of the Early Cretaceous global geography in lower to middle latitudes when angiosperms first appear (Parrish *et al.*, 1982; Hallam, 1984; Ziegler *et al.*, 1987, 2003; Barron *et al.*, 1994; Brenner, 1996; Doyle, 1999; Beerling & Woodward, 2001; Ibrahim, 2002). Examples of these wet regions with ancient angiosperm pollen records included rapidly changing rift zones as well as some coastal areas in northern and northwestern Gondwana (northern South America, parts of West Africa, the Middle East; Doyle *et al.*, 1982; Brenner, 1996). Also, coastal margins flanked by nearby or distant low-elevation mountains from southern Laurasia (Eastern North America, Britain) and far eastern Laurasia (Southeast Asia) have reconstructed wet climates and bear ancient angiosperm pollen grains (Ziegler *et al.*, 1987; Hughes, 1994; Doyle, 1999; Zhang, 1999). How these Early Cretaceous mesic regions compare hydrologically to those inhabited by basal angiosperms today requires further study.

However, other paleoenvironmental interpretations of the Early Cretaceous angiosperm pollen record indicated that chloranth fossil types also occurred in dry paleogeographic regions or regions with fluctuating dry/wet conditions that were as old as the wet assemblages in the approximate stratigraphic error of the pollen outcrops and cores (at least ± 5 Ma; Brenner, 1976; Doyle *et al.*, 1977; Doyle, 1999; Heimhofer *et al.*, 2005, 2007, 2008; Hochuli *et al.*, 2006). Not only do chloranths occur in xeric interpreted sediments, but also early angiosperm pollen diversity and abundance were as high or even higher than pollen assemblages inferred to be from wet conditions (Doyle, 1999). Dry environments for Early Cretaceous angiosperm pollen were inferred from the co-occurrence of rare early angiosperm pollen grains with abundant nonangiosperm taxa believed to reliably signal dry condition and lithological indicators of xeric climates (evaporites; Brenner, 1976; Doyle *et al.*, 1977; Doyle, 1999). Also, global scale stable isotopic proxies for temperature and moisture point to dry regional hydrology for some pollen assemblages (Brenner, 1976; Doyle *et al.*, 1977; Doyle, 1999; Heimhofer *et al.*, 2005, 2007; Hochuli *et al.*, 2006).

Whereas other angiosperms in these pollen cores may be xeric-adapted (see succeeding discussion, Doyle, 1999), we believe that the existence of ancient, nonextant drought-tolerant chloranths was unlikely. There are several taphonomic reasons favoring such a conclusion for Chloranthaceae as well as for probably most other basal angiosperm lineages. In offshore and coastal-deltaic environments that form the taphonomic window for the earliest angiosperm pollen assemblages currently available, three major taphonomic constraints are likely to apply: (i) transport of pollen from other nonrepresented habitats is likely an important process considering that angiosperm grains are rare in abundance and many extant basal most angiosperms rely partly or completely on wind pollination as well as occur in fluvial zones (Todzia, 1988; Hoorn, 1997; Kwit *et al.*, 1998; Endress, 2001; Bernhardt *et al.*, 2003; Thien *et al.*, 2003, 2009; Feild *et al.*, 2004; Endress & Doyle, 2009), (ii) pollen samples may time average ecological dynamics by compressing multiple communities and/or vegetation shifts associated geologically rapid wet and dry cycles within the sediment layers resolvable in Cretaceous pollen cores (Hoorn, 1994; van der Hammen & Hooghiemstra, 2000; Bush *et al.*, 2004), (iii) pollen samples may spatially average large areas geographic areas with complex elevational and habitat variation near tropical coasts in the pollen samples available (see succeeding discussion).

Currently, none of these taphonomic processes can be ruled out or have constraints put on them for any specific Early Cretaceous pollen assemblage. However, analogs for most of these complexities exist in much younger fossil sediments involving *Hedyosmum* (Chloranthaceae) pollen in South America or can be seen in the extant ecologies of extant basal angiosperms. Overall, these complexities suggest that the existences of wet-adapted angiosperms in otherwise xeric

pollen assemblages are likely. As an example of transport, small amounts of *Hedyosmum* pollen were found deposited in alluvial fan sediments of the Amazon River as well as other smaller lowland riparian deposition zones 50 km to more than 1000 km away from the montane cloud forest populations (Hoorn, 1997). Also, Pleistocene pollen spectra have demonstrated that dramatic glacial and interglacial vegetation shifts that involve large changes in *Hedyosmum* abundance or local extinction as wet forests shift to dry communities and back again would be time-averaged in sediment layers dated at resolution of $\pm 10\,000$ years (Hoorn, 1994; van der Hammen & Hooghiemstra, 2000; Bush *et al.*, 2004). For comparison, the temporal dynamics resolvable in sediment layers present in the Early Cretaceous record may be ± 1 Ma at best (Heimhofer *et al.*, 2005, 2007).

Also, habitat complexities among extant basal angiosperms also blunt the accuracy of pollen-based paleoecophysiological inferences. Several species, when viewed at a regional scale, would indicate that these species occurred in hydrological envelopes that are much drier than their actual ones (Fig. 1). Populations of some chloranths as well as other basal angiosperms occur in evaporative pre-montane or lowland tropical regions with lower annual rainfalls and strong monsoonal seasonality than their relatives found in cooler, everwet uplands (Todzia, 1988; Kwit *et al.*, 1998; Foster, 2001; Del-Val *et al.*, 2006). Field observations on all of these habitat disjuncts, however, reveal these species occur in microsites where local conditions override regional climate in producing much wetter habitats in the midst of highly evaporative regions. Such wet pockets include spring-fed stream banks and seeps in ravines that are shaded by dense forest cover (Kwit *et al.*, 1998; Feild *et al.*, 2004).

The formation of localized orographic cloud decks at low elevations near oceanic coastlines represents another local process that can create pockets of cloud forest on the tops of low mountains that are surrounded by arid scrublands or tropical dry forests (Foster, 2001; Del-Val *et al.*, 2006). If such ecologies were present in the Early Cretaceous, then in a single, large pollen catchment their drought intolerance would be obscured by more dominant signals of xeric conditions. In future work to assess taphonomic effects, it would be desirable to attempt to estimate the paleogeography of pollen capture catchments including size, local paleoaltimetry, and prevailing paleowinds based on climate modeling, as well as fluvial sedimentation rates to estimate time averaging. Although many of these variables will be difficult to parameterize, it may be possible to model different scenarios to resolve specific pollen assemblages that taphonomy influences the most.

However, the taphonomic complexities discussed in previous discussion to argue for the wet-adapted angiosperms as 'contaminants' in xeric sediments could be just as easily turned around to argue for rare, xeric angiosperms in wet-interpreted sediments. Also, because all of the earliest angiosperm fossil pollen deposits known occur within the stratigraphic error of

each other (± 5 Ma), it is difficult to argue on evolutionary direction of ecological evolution on the basis of fossil diversity and abundance alone (Feild *et al.*, 2004). Thus, the phylogenetic affinities of the fossil species in pollen assemblages may be the best guides to early angiosperm paleoecology. Extant angiosperm phylogeny strongly supports the hypothesis that evolution was most likely in the direction of wet to dry for angiosperms in general (Fig. 1; Jordan *et al.*, 2008). However, this is not to say that some of the xeric pollen assemblages that had xerophobic chloranthids did not contain xeric-adapted angiosperms. For example, *Walkeripollis*, a likely insect-pollinated magnoliid represented by a few grains and phylogenetically placed on the stem line related to Winterales, was identified from one xeric assemblage (Doyle, 1999). The winteroid stem lineage appears to have been more xeric during its early evolution than would be suggested by the modern species dominance of drought intolerant Winteraceae species in the Winterales crown group (Doyle, 1999). Since these possibly dry-adapted magnoliids are more nested in the phylogeny, it applies that these sediments are likely younger than wet-hypothesized sediments.

Leaning on the systematic affinities of pollen grains, analogies to extant deposition circumstances that are 130 Ma removed from these events, and variables that are difficult to quantify will always have an air of special pleading. However, speculations about the paleophysiology of water use by the parent plants that produced the chloranth pollen and perhaps other species are testable. The stable carbon isotopic compositions of leaf tissue ($\delta^{13}\text{C}_{\text{leaf}}$) of twenty species of Chloranthaceae as well as *Amborella* and Austrobaileya sampled from their natural wet forest habitats reveal highly characteristic signatures of low water use efficiency. Values of $\delta^{13}\text{C}_{\text{leaf}}$ are very depleted in ^{13}C , varying from -28‰ to -33‰ (Feild & Arens, 2007). These isotopic signatures suggest that these plants experience very little water limitation for leaf gas exchange. In contrast, angiosperm xerophytes exhibit much less negative isotopic signatures. Because $\delta^{13}\text{C}_{\text{leaf}}$ signatures and those of pollen can correspond (Jahren, 2004; Descolas-Gros & Scholzel, 2007), it could be possible to discern whether or not Early Cretaceous pollen grains originated in an everwet environment once the CO_2 source signal of Cretaceous atmospheric $\delta^{13}\text{C}$ is accounted for (see Jahren *et al.*, 2008). These measurements are now feasible on single pollen grains (Nelson *et al.*, 2007). However, first studies on extant basal angiosperms are needed to test how the $\delta^{13}\text{C}$ of pollen relates to leaf water use and in relation to pollination mode.

XEROPHOBIA AND EARLY ANGIOSPERM DIVERSIFICATION

Rather than weedy ruderals that immediately squeezed out the rest of the Mesozoic competition, a hypothesis of early angiosperm xerophobia suggests that life in the everwet zone permitted a phase of morphological experimentation that first

involved limited species diversity and low ecological abundances. Such a conclusion is reflected in the limited diversity of ecological strategies and abundance of basal most lineages today as well as the scarcity of angiosperm fossils during the first 10–15 Ma of their evolution (Heimhofer *et al.*, 2005, 2007). Based on the hypothesized ecology of the earliest angiosperms as xerophobes, a new view of the key innovations underpinning early angiosperm success can be posited. For example, no longer can simply the evolutions of vein branching in the leaves, wide vessels in the wood, and carpel closure in the flowers be viewed as the traits responsible for massive ecological rise of the angiosperms (Feild & Arens, 2005). Instead and as we have emphasized in the previous discussion, co-options of these traits from their ancestral roles for new functions, which hinged upon the evolution of less obvious innovations that modified these structures in relation to the rest of the plant, likely provided the keys to tremendous morphological diversity and abundance of angiosperms. These shifts may have involved a series of intermediate stages, after which the functions in the vascular and floral systems became elevated to the high productivities and efficiencies seen across the vast majority of derived angiosperms today (Bond, 1989; Brodribb & Feild, 2000; Feild *et al.*, 2004; Feild & Arens, 2005, 2007; Hacke *et al.*, 2006; Sperry *et al.*, 2007; Williams, 2008). Examples of such traits, which we believe require future inquiry, include evolution of vein density, evolution of simple perforation plates, evolution of the hydration of flowers by the phloem, as well as shortening of the fertilization interval for sexual reproduction (Williams, 2008). In evaluating the relevance of candidate key innovations for early angiosperm evolution, we discuss in the succeeding discussion how considerations of the community and environmental contexts of innovations are critical, but currently unexplored, dimensions.

Community context

A Mesozoic community context is essential for resolving when and how angiosperms achieved functional performances that conveyed advantages over their co-occurring nonangiosperm competitors (Feild & Arens, 2005, 2007). As one example, we can consider the key innovation of reticulated complex venation in the leaves. Of all the nonangiosperm clades known to have very angiosperm-like venation systems, only the dipterid ferns, which extend to the Triassic to Present (Stockey *et al.*, 2006) appear to have been coeval with Early Cretaceous angiosperms. Although estimates are unknown for Early Cretaceous fossil dipterid leaves, *Dipteris conjugata* from New Caledonia has moderately high vein densities and light-saturated photosynthetic rates that are greater than the co-occurring basal angiosperms *Amborella trichopoda*, *Ascarina solmsiana*, and *Trimenia neocaldeonia* (Table 2; vein density = 5.23 ± 0.12 SD mm mm^{-2} and $A_{\text{max}} = 10.2 \pm 0.1$ SD $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; T. J. Brodribb and T. S. Feild, field observations, 2007, $n = 7$ for each mean). Thus, all else being equal, the

evolution of complex reticulated veins would seem to be a neutral character for differentiating the early success of angiosperms as another preexisting clade already achieved it. However, observations of the local community context in extant sites where *Dipteris* and basal most angiosperm species overlap reveal ecological differentiation. For example in New Caledonia, no individuals of *D. conjugata*, which occurs out on landslip soils, would actually compete with an extant basal most angiosperms in the forest understory (T. S. Feild, personal observations, 2008).

These observations suggest that evaluating the evolutionary ecophysiological importance of reticulated veins is community context dependent. Because no nonangiosperms with complex reticulated veined are apparently known from forest understory habitats that the earliest angiosperms were hypothesized to have radiated in first, then the development of this venation system was possibly a novel functional advantage in the Early Cretaceous understory compared with other plants that early angiosperms may have competed with in the harvesting sunflecks (see previous discussion). In such a case, some trait advantages may depend on contingencies of being in the right context at the right time. Extending the vein example further, shade-adapted nonangiosperms with complex veined leaves were either much older or they may evolved complex venation after the major rise of angiosperms to dominance (i.e. *Gnetum*, Polypodiaceae; Boyce, 2005; Feild & Balun, 2008). Obtaining such fine-scale ecological information will be difficult, but it is essential if we understand how the functional traits influenced the invasion of angiosperms into diverse Mesozoic plant communities and began their ascent. Currently, pollen is our only coarse guide to community ecology for the earliest angiosperms. However, even functional segregation of fossils within a deposition environment, such as scoring plants for vein density or stomatal traits, may allow a parsing out of which species interacted with each other (Coifford *et al.*, 2006, 2007).

Environmental context

Understanding of any macro-radiation remains incomplete without consideration of the environmental context that clades and their novel innovations were evolving in (Feild & Arens, 2005). Previous opinions of early angiosperm evolution have generally rejected the role of Mesozoic environment or concluded that environmental change had a minor hand in early angiosperm evolution (Stebbins, 1974; Doyle & Donoghue, 1986, 1993; Taylor & Hickey, 1996; Crepet, 2008). However, as a possible xerophobic diversification occurring during a predominantly xeric geological interval (Parrish *et al.*, 1982; Hallam, 1984; Ziegler *et al.*, 1987; Moore *et al.*, 1992; Zharkov *et al.*, 1998; Rees *et al.*, 2000; Donnadieu *et al.*, 2006), then the environmental influences on water availability deserve careful consideration as processes influencing the pace and geographic spread of the early angiosperm radiation. Indeed, global environmental conditions that influence water supplies over several

scales, such as global temperatures, oceanic currents, land area extent, and atmospheric composition, were hardly quiescent during the Late Mesozoic because of a variety tectonic and igneous processes (Ziegler *et al.*, 1987; Moore *et al.*, 1992; Zharkov *et al.*, 1998; Rees *et al.*, 2000; Jahren *et al.*, 2001; Leckie *et al.*, 2002; Berner, 2006; Donnadieu *et al.*, 2006; Fletcher *et al.*, 2008).

The current evidence from the early angiosperm pollen fossil records that have the best stratigraphic resolution (on the order of a 1-Ma) so far suggest that diversification or abundance of the clade did not respond positively to global environmental instability (Heimhofer *et al.*, 2005; Hochuli *et al.*, 2006). The finest time resolution possible may be necessary because some of the candidate key environmental opportunities for angiosperms, such as atmospheric CO₂ changes, played out geologically rapidly (Weissert & Lini, 1991; Jahren *et al.*, 2001). The best stratigraphically constrained pollen assemblage sequences currently available come from late Barremian to late Aptian intervals of the Potomac Group as well as the Western and Algarve basins in Portugal (Doyle & Robbins, 1977; Heimhofer *et al.*, 2005, 2007, 2008; Hochuli *et al.*, 2006) that were situated at mid-paleolatitudes of Southern Laurasia. All other pollen sequences, despite the importance of these data in understanding the ancient geographic spread of angiosperms, are too coarsely resolved in time (see previous discussion; Crane & Lidgard, 1989; Lupia *et al.*, 1999). Also, these sediments are too young to capture the most ancient phases of angiosperms, and other older sediments from lower paleolatitudes imply that at least a few angiosperms had already acquired xeric ecologies by the Aptian (Brenner, 1976, 1996; Doyle *et al.*, 1977, 1982; Hickey & Doyle, 1977; Doyle, 1999). Nonetheless, angiosperms in these sediments are only represented by monosulcates many of which have been identified as chloranth, magnoliids, and basal monocots that precede the arrival of eudicots by 10 Ma (Heimhofer *et al.*, 2005). This indicates these sediments represent important records to understand how chloranth, basal monocots, and magnoliids responded to global climate change.

In the Early Cretaceous southern Laurasian sediments, the diversity and abundance of angiosperm monosulcates remained at low levels during the late Barremian to Aptian, a geological interval that included two worldwide Oceanic-Anoxic Events (OAE) that rapidly increased global CO₂ concentrations (pCO₂) (Heimhofer *et al.*, 2005; Hochuli *et al.*, 2006). Also, intermittently dry periods occurred in these regions (Ruffell & Batten, 1990; Heimhofer *et al.*, 2005, 2008; Steuber *et al.*, 2005). Although the time resolution of angiosperm pollen diversity samples would need to be increased to definitively resolve possible finer environment-diversity coevolution, the early angiosperm pollen diversification patterns currently available suggest that pulses of increased pCO₂ and fluctuating wet-dry conditions may have suppressed the radiation of angiosperms in this region since angiosperm diversity is low (Heimhofer *et al.*, 2005). An inability of dry precipitation regimes and pulses of increased pCO₂

by OAEs to influence diversification or spur a significant rise in abundance of early angiosperms is consistent with the hypothesis that that early angiosperms represented in the late Barremian-Aptian pollen samples from of Southern Laurasia were predominantly limited by water availability.

The xerophobia hypothesis may perhaps remove a previous environmental paradox of early angiosperm evolution. When the earliest angiosperms were hypothesized to be arid-adapted and as evolving their emblematic features in association with drought, an ecological difficulty was why the angiosperm line did not erupt into high diversity and abundance at the zenith of global drought-affected land area during the Late Jurassic through to earliest Cretaceous (Stebbins, 1965, 1974, 1976; Axelrod, 1972; Doyle & Hickey, 1976; Hickey & Doyle, 1977). A hypothesis of the earliest angiosperms as ecophysiological requiring everwet conditions resolves this contradiction since global drought would constrain the earliest angiosperms to wet areas which were of limited geographic extent during the Late Jurassic to Early Cretaceous (Ziegler *et al.*, 1987; Rees *et al.*, 2000). If xerophobia is a deep-seated ecophysiological requirement of angiosperms, then the emergence of global environmental stability associated with spread of extensive belts of high precipitation and humidity across the mid to Late Cretaceous resulting from high sea levels, extensive continental break-up and high global CO₂ with the massive phases of angiosperm diversification may be more than just a coincidence (Leckie *et al.*, 2002). Besides the extant ecological distribution data of basal angiosperms (Fig. 1), additional evidence for a major role of high water availability as a factor in early angiosperm evolution comes from the observation that resistive and cavitation-prone long, multiple pit-barred scalariform perforation plates (more than 40 bars) occur among many well-nested angiosperm eudicot clades (Carlquist, 2001a, 2003; Sperry *et al.*, 2007). Thus, an increase in the geographic extent of humid environments appears likely to have influenced angiosperm diversification by increasing the opportunities for population migration and isolation as wet adapted angiosperms for the first time migrated across diverse geographic heterogeneity (Stebbins, 1974; DiMichele & Aronson, 1992; Donoghue, 2008).

One remaining enigma is why the angiosperms did not originate in boreal zones that were present throughout the Mesozoic where wetter conditions would be most likely to prevail based on the distributions of coals and paleoclimate modeling results during the arid worlds of the Late Jurassic to earliest Cretaceous (Ziegler *et al.*, 1987; Rees *et al.*, 2000). Based on immense evidence, the early angiosperm radiation clearly began to spread from paleoequatorial tropical regions (Hickey & Doyle, 1977; Crane & Lidgard, 1989; Lupia *et al.*, 1999). Thus, a cryptic early Cretaceous boreal radiation has probably not escaped detection in the fossil record. It may be that cold temperatures that produced freezing, prevented hypothesized early angiosperms xerophobes

from establishing in high latitudes zones during the earliest Cretaceous (Price *et al.*, 1998; Price, 1999; Rees *et al.*, 2000; Steuber *et al.*, 2005; Sellwood & Valdes, 2006, 2008). The reconstructions of large ice sheets, however, conflict with paleobotanical data from the high-latitude regions that xerophobic early angiosperms could have established in (Vakhrameev, 1991; Rees *et al.*, 2000). But this does not rule out winter high-latitude frosts during the globally cool periods of the earliest Cretaceous (Rees *et al.*, 2000).

Most extant basal most angiosperms are killed by frost in temperate and tropical montane environments (Martin & Ogden, 2005). Tolerance to cold winter temperatures (−30 °C) by deciduousness and winter dormancy occurs only in a few basal most angiosperms. Examples include *Schisandra chinensis* and *Chloranthus serratus* from Siberia as well as several Nymphaeales. But still it remains unclear why angiosperms reached coastal fringes of high-latitude regions so late since these would offer high rainfall, high humidity, and probably locally frost-free conditions. Indeed, the preservation of early angiosperms in these temperate maritime deposition sites during the mid-Cretaceous actually provided the evidence that angiosperms migrated later to poles of both hemispheres (Hickey & Doyle, 1977; Retallack & Dilcher, 1981, 1986; Cuneo & Gandolfo, 2005).

CONCLUSION

Integration of the current perspectives provided by ecophysiology, phylogeny, and paleobotany on the earliest angiosperms has identified water as a major resource constraining their initial success at a broad range of scales. As we have highlighted throughout our discussions, there are many more exciting avenues of future research on extant basal angiosperms to test the hypotheses posed here as well as expose additional hypotheses on the ancestral functions and transitions in early angiosperm biology. However, the most immediate need in the future is a comprehensive test of the xerophobia hypothesis because we still lack a clear understanding of the extent that extant basal most clades reflect early angiosperms. Although some preliminary indications suggest that extant basal angiosperms reflect some of the forms and functions of some the oldest fossil angiosperms currently known, testing the xerophobia hypothesis will require new multidisciplinary research approach necessitating new collaborations among phylogeneticists, geologists, paleobotanists, paleoclimate modelers, and plant physiologists to determine the nature of the ecophysiological watermarks on early angiosperm form, function, and habitats during the Early Cretaceous. Our analysis here leaves us with a final thought. A preliminary reading of the early angiosperm diversification suggests that rather than weedy rudists that immediately squeezed out the rest of the Mesozoic competition, the early angiosperm line may have been a rare, localized line that was highly dependent upon water for several million years.

With the widespread drought in the Jurassic and the earliest Cretaceous, the line may have just scrapped by in wet patches. However, with a tectonically based change of fortune, humidity rose and wet environments expanded and angiosperms may ultimately been able to dominate the plant world.

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